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RESEARCH ARTICLE

SYNTHESIS, MICROBIOLOGICAL EVALUATION AND MT-QSAR OF 3, 4-DIMETHOXY BENZOIC ACID DERIVATIVES

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ABSTRACT

In the present work, novel derivatives of veratric acid (V_1- V_{34}) were synthesized and tested *in vitro* for their antibacterial activity against Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative bacteria *Escherichia coli* as well as antifungal activity against fungal strains *Candida albicans* and *Aspergillus niger* by standard serial dilution method using ciprofloxacin and fluconazole as reference compounds in case of antibacterial and antifungal activity respectively. The IR, ^{1}H NMR and ^{13}C NMR spectral data of the synthesized compounds were found in agreement with the assigned molecular structures. The investigation of antimicrobial screening data revealed that most of the synthesized compounds demonstrated good antimicrobial activity against all bacteria and fungi used in the study. Compounds (V_2, V_{21}, V_{25}, V_{28}) demonstrated most significant activity against *C. albicans* (MIC=6.25 µg/ml). The outcome of the study suggested that the test compound V_{28} may be utilized as potential antimicrobial agent against *S. aureus, B. subtilis, E. coli, C. albicans and A. niger*. *mt*-QSAR investigation with linear regression analysis was applied to find the relationship between chemical structures and biological activities of a series of analogs quantitatively. *mt*-QSAR studies revealed that antimicrobial activity of these synthesized derivatives against microorganisms under test mainly governed by topological parameters [valence zero order molecular connectivity index ($^0\chi^V$), kier's alpha second order shape index ($\kappa\alpha_2$)] and electronic parameter [total energy (Te)].

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INTRODUCTION

The inevitable consequence of the widespread use of antimicrobial agents has been the emergence of antibiotic-resistant pathogens, fueling an ever-increasing need for new drugs (Hardman *et al.,* 2001). During the past years, an increasing interest has been devoted to the study of new and more selective antimicrobial agents. Due to this, not only new synthetic methods have been developed, but a greater amount of interest has been devoted to comprehension of their mechanism of action and structure activity relationship (Maccioni *et al.,* 2002). Developing new antimicrobial agents are among the most dramatic examples of the advances of modern medicine. Many infectious diseases once considered incurable and lethal are now amenable to treatment with a few pills. The remarkably powerful and specific activity of antimicrobial drugs is due to their selectivity for targets that are either unique to microorganisms or much more important in them than in humans. Among these targets are bacterial and fungal cell wall-synthesizing enzymes, the bacterial ribosome, the enzymes required for nucleotide synthesis, DNA replication and the machinery of viral replication (Katzung *et al.,* 2007). Antibacterial resistance is a growing problem which necessitates the discovery of newer antibiotics with activity against resistant strains (Kumar *et al.*, 2010).

Veratric acid isolated from the stem bark of *Tabebuia impetiginosa* (Awale *et al.,* 2005) has been reported to have antibacterial (Malarczyk *et al*., 2000), antifungal (Zemek *et al*., 1987), antioxidant (Szwajgier *et al.*, 2005), anti-inflammatory (Zheng *et al.*, 2002), antihypertensive (Raja *et al.*, 2011) and antispasmodic activities (Sommers *et al.,* 1997). Simple organic acids are having good antimicrobial potential as evidenced by our previous research publications which describe the synthesis and antimicrobial properties of derivatives of simple organic acids viz. sorbic acid (Nararasimhan *et al*., 2003), cinnamic acid (Narasimhan *et al.*,

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2004), anacardic acid (Narasimhan *et al.*, 2006), myristic acid (Narasimhan *et al.*, 2006), caprylic acid (Chaudhary *et al.*, 2008), anthranilic acid (Mahiwal *et al.*, 2012) and dodecanoic acid (Sarova *et al.*, 2011). Veratric acid is used as intermediate for pharmaceutical (especially for antipyretic, analgesic, anti-rheumatism) and other organic synthesis. They are used as matrix for ionization of peptides, proteins and carbohydrates. Prazosin, a peripheral vasodilator and antihypertensive, is also an example of the application of veratric acid (Raja *et al.*, 2011). It plays an important role in producing antibiotics and various dyes.

Quantitative structure activity relationship (QSAR), one of the most important areas in chemistry, gives information that is useful for drug design and medicinal chemistry (Hansch *et al.*, 1964). QSAR is a mathematical model that relates a quantitative measure of chemical structure to a biological effect. Thus, the structure-activity relationship of the molecules could be explained quantitatively (Marzio *et al*., 2004). A QSAR study describes a definite role in a quantitative term of a structural feature in the molecule with a definite contribution to the activity of a particular physicochemical property of the structural feature. These chemical descriptors, which include parameters to account for hydrophobicity, electronic, inductive, or polar properties, and steric effects, are determined empirically or by calculations. Thus, QSAR studies have a predictive ability and simultaneously provide deeper insight into the mechanism of drug receptor interactions (Vasanthanathan *et al.*, 2006).

In the present study, we attempted to develop three different types of multi target QSAR (*mt*-QSAR) models *viz. mt*-QSAR model for describing antibacterial activity of synthesized compounds against *S. aureus, B. subtilis* and *E. coli*, *mt*-QSAR model for describing antifungal activity of synthesized compounds against *C. albicans* and *A. niger* as well as a common *mt*-QSAR model for describing the antimicrobial (overall antibacterial and antifungal) activity of synthesized compounds by calculating their average antibacterial activity, antifungal activity and antimicrobial activity values. The significant *in vitro* antimicrobial activity of esters and amides/anilides derivatives of veratric acid and their QSAR studies which indicated the importance of topological parameters in describing the antimicrobial activity has already been reported by (Narasimhan *et al.*, 2009). We have previously reported the synthesis, antimicrobial evaluation and QSAR studies of some simple organic acid derivatives as possible antimicrobial agents as a part of our composite programme on rational drug design (Narasimhan *et al.,* 2007; Narasimhan *et al.,* 2007; Narasimhan *et al.,* 2007; Gangwal *et al.,* 2003; Narasimhan *et al.,* 2006; Narasimhan *et al.,* 2006; Narasimhan *et al.,* 2006; Kumar *et al.,* 2007; Narasimhan *et al.,* 2007; Narasimhan *et al.,* 2007).

Schiff bases are considered to be among the most important group of compounds in medicinal chemistry due to their preparative accessibility, structural variety and wide biological profile (Sigroha *et al.*, 2012). Keeping this observation in mind and in continuation of our study in the field of antimicrobial evaluation and QSAR studies (Kumar *et al.*, 2010, 2012; Judge *et al.,* 2012, Narang *et al*., 2012), we hereby report the synthesis, antimicrobial evaluation and *mt*-QSAR studies of veratric acid derivatives. In order to bring into sharper focus the fundamental issue of the activities of different derivatives of veratric acid and in pursuit of achieving this goal, our research efforts focused on the synthesis, antimicrobial, and QSAR studies (Kumar *et al.*, 2010, Kumar *et al*., 2010, Judge *et al*., 2011) herewith we report the synthesis of different novel derivatives of veratric acid (schiff bases and benzohydrazides) and evaluate their *in vitro* antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi in order to possess moderate to good antimicrobial activity of title compounds in comparison to the parent compound. Further, we have decided to carry out the *mt*-QSAR studies to perceive the importance of molecular properties, which are critical in accentuating the antimicrobial activity of veratric acid derivatives (Narasimhan *et al*., 2009).

MATERIALS AND METHODS

Veratric acid was purchased from HIMEDIA Laboratories Pvt. Ltd., Mumbai, India. The other reagents and solvents used were of analytical grade. Melting points of synthesized compounds were determined in open capillary using Decibel melting point apparatus and recorded in ^{o}C without correction. Thin layer chromatographic analysis of compounds was performed on silica gel G coated glass plates. The mobile phases were selected according to the polarity of the compounds. The spots were visualized by exposure to iodine vapour and the formation of final product was ascertained by single spot on TLC plate using Toluene: Chloroform (7:3) as solvent system. The infrared spectra for the synthesized compounds were recorded by BRUKER ECO ATR spectrophotometer. Proton nuclear magnetic resonance spectra (^{1}H NMR & ^{13}C NMR) were recorded on Bruker Avance II 400 NMR Spectrophotometer using TMS (Chemical shift δ in ppm) as an internal standard. ^{1}H NMR and ^{13}C NMR spectra were recorded with DMSO as a solvent.

General procedure for synthesis of ester of veratric acid

Veratric acid (0.08 mol) was refluxed with (0.74 mol) ethyl alcohol in the presence of few drops of sulphuric acid for 3-4 h. The reaction progress was monitored by TLC using Toluene: Chloroform (7:3) as solvent system and iodine as visualizing agent. After the completion of reaction, the reaction mixture was poured in 200 ml ice cold water and the ester formed was extracted with ether (50 ml). The ether layer was separated and on evaporation yielded the white crude ester which was then recrystallized from ethanol. Finally pure 12.66 g (75.35 %) of ethyl ester of veratric acid was obtained.

General procedure for synthesis of veratric acid hydrazide

Ethyl veratrate (3.15 g, 0.015 mol) was taken in 100 ml round bottom flask, and then hydrazine hydrate (0.75 g, 0.015 mol) was added and refluxed for 3-4 h till the completion of reaction. Confirmation of the final product was monitored by TLC. The reaction mixture was then cooled and the precipitates were separated by filteration. The crude product was recrystallized from ethanol to get pure 2.54g (87.58 %) crystals of veratric acid hydrazide.

General procedure for synthesis of schiff bases of veratric acid (V_1- V_{21})

Veratric acid hydrazide (9.80 g, 0.05 mol) dissolved in 50 ml ethanol was refluxed with different aldehydes (0.05 mol) in ethanol for 5 h. On the completion of reaction, the reaction mixture was poured on ice cold water at room temperature and the crude precipitates were filtered, dried and recrystallized from ethanol.

General procedure for benzohydrazide derivatives of veratric acid (V_{22}- V_{34})

Veratric acid hydrazide (0.05 mol) synthesized above was refluxed with chloroacetyl chloride (0.01 mol) in the presence of glacial acetic acid in absolute ethanol for 8-10 h. When the reaction had been completed, the reaction mixture was cooled in ice cold water. The resultant precipitates were filtered, washed with excess water, concentrated under reduced pressure and recrystallized with ethanol. The solid compound synthesized above was allowed to react with different corresponding anilines (0.01mol) and refluxed for 10-15 h in the presence of few drops of glacial acetic acid in absolute ethanol (20 ml) to synthesize benzohydrazide derivatives. After the completion of reaction, the reaction mixture was added to 200 ml ice cold water and the yielded precipitates were filtered, washed with water, vacuum dried and recrystallized by ethanol to get the final products.

3,4-dimethoxy-N'-methylenebenzohydrazide (V_1):
Yield – 72.11%; Mp (°C) 188-190 ˚C; R_f value – 0.40; IR (cm^{-1}): 2942.28 (C-H str., -OCH_3), 3027.82 (C-H str., aromatic), 1558.62 (C=C str., aromatic), 693.82 (C-C out of plane bending, aromatic), 799.84 (C-H out of plane bending, aromatic), 869.78 (C-H deformed, aromatic), 1645.45 (C=N str.), 3085.98 (N-H str.,2° amide), 1602.60 (N-H in plane bending, 2°amide), 1645.45 (C=O str., 2°amide); ^{1}H-NMR (δ (ppm): 3.221 (s, 6H, -OCH_3), 7.023-7.342 (m, 3H, Ar-H), 8.204 (s, 1H, -NH); ^{13}C-NMR (δ (ppm) 162.5 (C=O, CONH), 151.8 (CH_2, N=CH_2), 155.4 (C, C-4), 145.7 (C, C-3), 128.3 (C, C-1), 122.9 (C, C-6), 117.7 (C,C-5), 115.5 (C, C-2), 58.1 (CH_3, OCH_3); Mass (m/z): 208 (M^+); Elemental analysis: Calculated: C, 57.68%; H, 5.81%; N, 13.45%; Found: C, 57.28%; H, 5.61%; N, 13.15%.

N'-(4-nitrobenzylidene)-3,4-dimethoxybenzohydrazide (V_2):
Yield – 78.02%; Mp (°C) 194-200 ˚C; R_f value – 0.52; IR (cm^{-1}): 2945.48 (C-H str., -OCH_3), 3012.00 (C-H str., aromatic), 1457.23 (C=C str., aromatic), 684.55 (C-C out of plane bending, aromatic), 745.79 (C-H out of plane bending, aromatic), 850.51 (C-H deformed, aromatic), 1635.79 (C=N str.), 3545.86 (N-H str., 2° amide), 1635.79 (N-H in plane bending, 2°amide), 1635.79 (C=O str., 2°amide), 1361.83 (Ar-NO_2 str.). ^{1}H-NMR (δ (ppm)): 3.368 (s, 6H, -OCH_3), 7.438-7.765 (m, 3H, Ar-H), 8.321 (s, 1H, -NH), 8.564 (s, 1H, -CH); ^{13}C-NMR (δ (ppm) 163.5 (C=O, CONH), 151.3 (C, C-4), 152.6 (C, C-4'), 147.5 (C, C-3),145.5 (CH, N=CH ($C_6H_4NO_2$), 138.7 (C, C-1'), 132.4 (C, C-2', C-6'), 129.1 (C, C-1), 123.4 (C, C-3', C-5'), 123.9 (C, C-6), 118.3 (C, C-5), 111.6 (C, C-2), 57.2 (CH_3, OCH_3); Mass (m/z): 329 (M^+); Elemental analysis: Calculated: C, 58.36%; H, 4.59%; N, 12.76%; Found: C, 58.06%; H, 4.39%; N, 12.36%.

N'-(2-nitrobenzylidene)-3,4-dimethoxybenzohydrazide (V_3):
Yield – 89.82.11%; Mp (°C) 176-180 ˚C; R_f value – 0.47; IR (cm^{-1}): 2975.16 (C-H str., -OCH_3), 3024.34 (C-H str., aromatic), 1547.37 (C=C str., aromatic), 669.06 (C-C out of plane bending, aromatic), 786.05 (C-H out of plane bending, aromatic), 845.63 (C-H deformed, aromatic), 1691.96 (C=N str.), 3116.15 (N-H str., 2° amide), 1630.74 (N-H in plane bending, 2°amide), 1649.64 (C=O str., 2°amide) , 1365.24 (Ar-NO_2 str.). ^{1}H-NMR (δ (ppm)): 3.876 (s, 6H, -OCH_3), 7.253-7.478 (m, 3H, Ar-H), 8.654 (s, 1H, -NH), 8.894 (s, 1H, -CH); ^{13}C-NMR (δ (ppm) 164.0 (C=O, CONH), 150.0 (C, C-4), 148.7 (C, C-3), 145.2 (C, C-2') 142.3 (CH, N=CH ($C_6H_4NO_2$), 136.8 (C, C-5'), 134.4 (C, C-4'), 132.4 (C, C-6'), 127.2 (C, C-1), 124.4 (C, C-1'), 121.1 (C, C-6), 120.0 (C, C-3'), 114.3 (C, C-5), 110.2 (C, C-2), 56.2 (CH_3, OCH_3); Mass (m/z): 329 (M^+); Elemental analysis: Calculated: C, 58.36%; H, 4.59%; N, 12.76%; Found: C, 58.16%; H, 4.29%; N, 12.36%.

N'-(3-methoxybenzylidene)-3,4-dimethoxybenzohydrazide V_4):
Yield – 58.75.%; Mp (°C) 120-122 ˚C; R_f value – 0.63; IR (cm^{-1}): 2935.42 (C-H str.,-OCH_3), 3045.78 (C-H str., aromatic), 1556.91 (C=C str., aromatic), 691.85 (C-C out of plane bending, aromatic), 790.63 (C-H out of plane bending, aromatic), 860.30 (C-H deformed, aromatic), 1659.64 (C=N str.), 3212.08 (N-H str., 2° amide), 1704.32 (N-H in plane bending, 2°amide), 1650.03 (C=O str., 2°amide); ^{1}H-NMR (δ (ppm)): 3.734 (s, 9H, -OCH_3), 7.672-7.877 (m, 3H, Ar-H), 8.012 (s, 1H, -NH), 8.478 (s, 1H, -CH); ^{13}C-NMR (δ (ppm) 162.2 (C=O, CONH), 161.1 (C, C-3'), 151.02(C, C-4), 147.6 (C, C-3), 141.7(CH, N=CH ($C_6H_4NO_2$), 135.6 (C, C-1'), 129.1 (C, C-1), 128.1 (C, C-5'), 120.4 (C, C-6'), 121.7 (C, C-6), 114.2 (C, C-4'), 113.2 (C, C-5), 112.4 (C, C-2'), 111.5 (C, C-2), 55.2 (CH_3, OCH_3); Mass (m/z): 314 (M^+); Elemental analysis: Calculated: C, 64.96%; H, 5.77%; N, 8.91%; Found: C, 64.56%; H, 5.47%; N, 8.71%.

N'-benzylidene-3,4-dimethoxybenzohydrazide (V_5): IR(cm^{-1}):
Yield – 67.12%; Mp (°C) 130-132˚C; R_f value – 0.75; 2943.75 (C-H str., -OCH_3), 3004.90 (C-H str., aromatic), 1513.75 (C=C str., aromatic), 690.04 (C-C out of plane bending, aromatic), 723.12 (C-H out of plane bending, aromatic), 857.53 (C-H deformed, aromatic), 1650.10 (C=N str.), 3198.44 (N-H str., 2° amide), 1720.94 (N-H in plane bending, 2°amide), 1650.10 (C=O str., 2°amide); ^{1}H-NMR (δ (ppm)): 3.194 (s, 6H, -OCH_3), 7.089-7.237 (m, 3H, Ar-H), 8.569 (s, 1H, -NH), 8.902 (s, 1H, -CH); ^{13}C-NMR (δ (ppm) 165.1 (C=O, CONH), 152.1(C, C-4), 148.6 (C, C-3), 144.0(CH, N=CH (C_6H_4), 132.2 (C, C-4'), 131.6 (C, C-1'), 129.4 (C, C-3', C-5'), 128.4 (C, C-2', C-6'), 126.1 (C, C-1), 121.3(C, C-6), 114.2 (C, C-5), 111.5 (C, C-2), 56.4 (CH_3, OCH_3); Mass (m/z): 284 (M^+); Elemental analysis: Calculated: C, 67.59%; H, 5.67%; N, 9.85%; Found: C, 67.39%; H, 5.37%; N, 9.65%.

N'-((furan-2-yl)methylene)-3,4-dimethoxybenzohydrazide (V_6):
Yield – 65.69.11%; Mp (°C) 118-120˚C; R_f value – 0.53; IR(cm^{-1}): 2838.55 (C-H str., -OCH_3), 2943.72 (C-H str., aromatic), 1513.29 (C=C str., aromatic), 656.43 (C-C out of plane bending, aromatic), 785.34 (C-H out of plane bending, aromatic), 845.81 (C-H deformed, aromatic), 1677.80 (C=N str.), 3217.59 (N-H str., 2° amide), 1677.80 (N-H in plane bending, 2°amide), 1641.00 (C=O str., 2°amide); ^{1}H-NMR (δ (ppm)): 3.702 (s, 6H, -OCH_3), 7.389-7.465 (m, 3H, Ar-H),

8.340 (s, 1H, -NH), 8.956 (s, 1H, -CH); ^{13}C-NMR (δ (ppm) 162.1 (C=O, CONH), 151.1(C, C-4), 150.2 (C, C-3), 147.6 (C, C-1'), 142.2 (C, C-4'), 135.6(CH, N=CH (C_4H_3O), 126.5 (C, C-1), 119.2 (C, C-6), 115.2 (C, C-2), 113.9 (C, C-5), 108.4 (C, C-3'), 107.5 (C, C-2'), 56.1 (CH_3, OCH_3); Mass (m/z): 274 (M^+); Elemental analysis: Calculated: C, 61.31%; H, 5.14%; N, 10.21%; Found: C, 61.71%; H, 5.54%; N, 10.01%.

N'-((2-hydroxynaphthalen-3-yl)methylene)-3,4-dimethoxybenzohydrazide (V_7): Yield – 74.15%; Mp (°C) 125-127 ˚C; R_f value – 0.42; IR(cm^{-1}): 2937.30 (C-H str., -OCH_3), 3082.12 (C-H str., aromatic), 1511.44 (C=C str., aromatic), 673.49 (C-C out of plane bending, aromatic), 760.41 (C-H out of plane bending, aromatic), 876.29 (C-H deformed, aromatic), 1656.10 (C=N str.), 3211.98 (N-H str., 2° amide), 1677.80 (N-H in plane bending, 2°amide), 1656.10 (C=O str., 2°amide); ^{1}H-NMR (δ (ppm)): 3.633 (s, 6H, -OCH_3), 7.653-7.774 (m, 3H, Ar-H), 8.006 (s, 1H, -NH), 8.500 (s, 1H, -CH); ^{13}C-NMR (δ (ppm) 162.5 (C=O, CONH), 156.8 (C, C-2'), 153.7 (C, C-4), 147.6 (C, C-3), 142.6 (CH, N=CH ($C_{10}H_6OH$), 136.2 (C, C-4'), 130.2 (C, C-9'), 129.3 (C, C-1), 129.2 (C, C-10'), 126.7(C, C-6'), 126.4 (C, C-8'), 123.2 (C, C-5'), 120.8 (C, C-7'), 118.2 (C, C-6), 114.7 (C, C-5), 113.3 (C, C-2), 107.2 (C, C-3'), 105.6 (C, C-1'), 56.9 (CH_3, OCH_3); Mass (m/z): 350 (M^+); Elemental analysis: Calculated: C, 68.56%; H, 5.18%; N, 8.00%; Found: C, 68.96%; H, 5.38%; N, 8.40%.

3,4-dimethoxy-N'-((E)-3-phenylallylidene)benzohydrazide(V_8): Yield – 55.08.%; Mp (°C) 217-219˚C; R_f value – 0.48; IR(cm^{-1}): 2975.02 (C-H str., -OCH_3), 3007.18 (C-H str., alkene), 903.94 (C-H bending, alkene), 3053.03 (C-H str., aromatic), 1536.55 (C=C str., aromatic), 691.18 (C-C out of plane bending, aromatic), 724.36 (C-H out of plane bending, aromatic), 854.82 (C-H deformed, aromatic), 1677.75 (C=N str.), 3248.83 (N-H str., 2° amide), 1677.75 (N-H in plane bending, 2°amide), 1649.81 (C=O str., 2°amide); ^{1}H-NMR (δ (ppm)): 3.466 (s, 6H, -OCH_3), 6.845-7.204 (m, 3H, Ar-H), 8.096 (s, 1H, -NH), 8.611 (s, 1H, -CH); ^{13}C-NMR (δ (ppm) 161.2 (C=O, CONH), 152.0 (C, C-4), 150.0 (C, C-3), 140.1 (CH, N=CH-CH=CH-C_6H_5, C-3'), 138.1 (CH, N=CH-CH=CH-C_6H_5, C-1'), 127.2 (CH, N=CH-CH=CH-C_6H_5, C-2'), 126.3 (C, C-1), 119.0 (C, C-6), 116.4 (C, C-5), 113.6 (C, C-2), 56.6 (CH_3, OCH_3); Mass (m/z): 310 (M^+); Elemental analysis: Calculated: C, 69.66%; H, 5.85%; N, 9.03%; Found: C, 69.36%; H, 5.45%; N, 9.33%.

N'-(3,4-dimethoxybenzylidene)-3,4-dimethoxybenzohydrazide (V_9): Yield – 84.5%; Mp (°C) 142-144 ˚C; R_f value – 0.69; IR (cm^{-1}): 2863.08 (C-H str., -OCH_3), 3005.92 (C-H str., aromatic), 1511.10(C=C str., aromatic), 695.00 (C-C out of plane bending, aromatic), 734.12 (C-H out of plane bending, aromatic), 862.92 (C-H deformed, aromatic), 1650.44 (C=N str.), 3194.45 (N-H str., 2° amide), 1619.96 (N-H in plane bending, 2°amide), 1650.44 (C=O str., 2°amide); ^{1}H-NMR (δ (ppm)): 3.417 (s, 12H, -OCH_3), 7.061-7.488 (m, 4H, Ar-H), 7.555-7.935 (m, 3H, Ar-H), 8.449 (s, 1H, -NH), 8.695 (s, 1H, -CH); ^{13}C-NMR (δ (ppm) -163.3 (C=O, CONH), 152.1 (C, C-4), 148.2 (C, C-3), 142.6 (CH, N=CH (C_6H_3(OCH_3)), 136.2 (C, C-4'), 130.2 (C, C-9'), 129.3 (C, C-1), 129.2 (C, C-10'), 126.7(C, C-6'), 126.4 (C, C-8'), 123.2 (C, C-5'), 120.8 (C, C-7'), 118.2 (C, C-6), 114.7 (C, C-5), 113.3 (C, C-2), 112.3 (C, C-2') 107.2 (C, C-3'), 105.6 (C, C-1'), 56.9 (CH_3, OCH_3); Mass (m/z): 344 (M^+); Elemental analysis: Calculated: C, 62.78%; H, 5.85%; N, 8.13%; Found: C, 62.38%; H, 5.95%; N, 8.43%.

N'-(4-methoxybenzylidene)-3,4-dimethoxybenzohydrazide(V_{10}): Yield – 77.73%; Mp (°C) 100-102 ˚C; R_f value – 0.32; IR(cm^{-1}): 2948.21 (C-H str., -OCH_3), 3057.58 (C-H str., aromatic), 1536.33 (C=C str., aromatic), 676.24 (C-C out of plane bending, aromatic), 758.34 (C-H out of plane bending, aromatic), 832.01 (C-H deformed, aromatic), 1692.03 (C=N str.), 3228.51 (N-H str., 2° amide),1692.03 (N-H in plane bending, 2°amide), 1658.92 (C=O str., 2°amide); ^{1}H-NMR (δ (ppm)): 3.265 (s, 6H, -OCH_3), 7.295-7.394 (m, 3H, Ar-H), 8.320 (s, 1H, -NH), 8.556 (s, 1H, -CH); ^{13}C-NMR (δ (ppm) 163.1 (C=O, CONH), 154.4 (C, C-4), 150.2 (C, C-3), 143.0 (CH, N=CH (C_6H_4 (OCH_3)), 125.9 (C, C-1), 121.9 (C, C-6), 116.3 (C, C-5), 113.1 (C, C-2), 55.9 (CH_3, OCH_3); Mass (m/z): 314 (M^+); Elemental analysis: Calculated: C, 64.96%; H, 5.77%; N, 8.91%; Found: C, 64.56%; H, 5.57%; N, 8.61%.

N'-(4-(dimethylamino)benzylidene)-3,4-dimethoxybenzohydrazide (V_{11}): Yield – 48.49%; Mp (°C) 234-236 ˚C; R_f value – 0.34; IR(cm^{-1}): 2929.32 (C-H str., -OCH_3), 3044.88 (C-H str., aromatic), 1547.58 (C=C str., aromatic), 659.71 (C-C out of plane bending, aromatic), 770.62 (C-H out of plane bending, aromatic), 813.86 (C-H deformed, aromatic), 1658.78 (C=N str.), 3241.52 (N-H str., 2° amide), 1611.97 (N-H in plane bending, 2°amide), 1658.78 (C=O str., 2°amide), 1181.70 (3°amine); ^{1}H-NMR (δ (ppm)): 3.950 (s, 6H, -OCH_3), 6.994-7.306 (m, 3H, Ar-H), 8.088 (s, 1H, -NH), 8.630 (s, 1H, -CH); ^{13}C-NMR (δ (ppm) 163.6 (C=O, CONH), 154.5 (C, C-4), 143.3 (CH, N=CH (C_6H_4N(CH_3)$_2$), 147.7 (C, C-3), 126.2 (C, C-1), 122.1 (C, C-6), 116.2 (C, C-5), 114.4 (C, C-2), 56.1 (CH_3, OCH_3), 40.2 (CH_3, N=CH ($C_6H_4N(CH_3)_2$); Mass (m/z): 327 (M^+); Elemental analysis: Calculated: C, 66.04%; H, 6.47%; N, 12.84%; Found: C, 66.37%; H, 6.77%; N, 12.54%.

N'-(2-chlorobenzylidene)-3,4-dimethoxybenzohydrazide (V_{12}): Yield – 35.84.11%; Mp (°C) 110-112 ˚C; R_f value – 0.77; IR(cm^{-1}): 2996.78 (C-H str., -OCH_3), 3028.57 (C-H str., aromatic), 1552.05 (C=C str., aromatic), 699.94 (C-C out of plane bending, aromatic), 780.91 (C-H out of plane bending, aromatic), 864.05 (C-H deformed, aromatic), 1658.89 (C=N str.), 3224.23 (N-H str.,2° amide),1612.02 (N-H in plane bending, 2°amide), 1658.89(C=O str., 2°amide), 757.16 (C-Cl str.); ^{1}H-NMR (δ (ppm)): 3.241 (s, 6H, -OCH_3), 7.065-7.345 (m, 3H, Ar-H), 8.112(s, 1H, -NH), 8.755 (s, 1H, -CH); ^{13}C-NMR (δ (ppm) 164.1 (C=O, CONH), 152.5 (C, C-4), 146.9 (C, C-3), 143.8 (CH, N=CH (C_6H_4Cl)), 133.5 (C-Cl, N=CH (C_6H_4Cl), 128.8 (C, C-1), 118.9 (C, C-6), 117.7 (C, C-5), 114.2 (C, C-2), 55.8 (CH_3, OCH_3); Mass (m/z): 319 (M^+); Elemental analysis: Calculated: C, 60.29%; H, 4.74%; N, 8.79%; Found: C, 60.59%; H, 4.44%; N, 8.69%.

N'-(2-methoxybenzylidene)-3,4-imethoxybenzohydrazide(V_{13}): Yield – 36.87.%; Mp (°C) 108-110 ˚C; R_f value – 0.54; IR(cm^{-1}): 2906.26 (C-H str., -OCH_3), 3097.05 (C-H str., aromatic), 1513.56 (C=C str., aromatic), 667.85 (C-C out of plane bending, aromatic), 751.95 (C-H out of plane bending, aromatic), 878.95 (C-H deformed, aromatic), 1640.10 (C=N str.), 3148.08 (N-H str., 2° amide), 1692.12 (N-H in plane

bending, 2°amide),1649.79 (C=O str., 2°amide); ^{1}H-NMR (δ (ppm)): 3.327 (s, 9H, -OCH_3), 7.006-7.168 (m, 4H, Ar-H), 7.394-7.607 (m, 3H, Ar-H), 8.818 (s, 1H, -NH), 8.937 (s,1H,-CH); ^{13}C-NMR (δ (ppm) 163.0 (C=O, CONH), 154.5 (C, C-4), 150.9 (C, C-3), 143.8 (CH, N=CH (C_6H_4O)), 129.3 (C, C-1), 121.7 (C, C-6), 116.2 (C, C-5), 111.9 (C, C-2), 56.9 (CH_3, OCH_3); Mass (m/z): 314 (M^+); Elemental analysis: Calculated: C, 64.96%; H, 5.77%; N; 8.91%; Found: C, 64.56%; H, 6.17%; N, 8.71%.

N'-(5-bromo-2-fluorobenzylidene)-3,4-dimethoxybenzohydrazide (V_{14}): Yield – 47..36%; Mp (°C) 132-134 ˚C; R_f value – 0.58; IR(cm^{-1}): 2967.97 (C-H str., -OCH_3), 3038.63 (C-H str., aromatic), 1503.17 (C=C str., aromatic), 668.00(C-C out of plane bending, aromatic), 764.41 (C-H out of plane bending ,aromatic), 873.24 (C-H deformed, aromatic), 1632.66, (C=N str.), 3108.38 (N-H str., 2° amide), 1720.85(N-H in plane bending, 2° amide), 1673.09 (C=O str., 2°amide), 1270.71 (C-F str.), 640.45 (C-Br str.) ; ^{1}H-NMR (δ (ppm)): 3.402 (s, 6H, -OCH_3), 7.267-7.429 (m, 3H, Ar-H), 7.504-7.750 (m, 3H, Ar-H), 8.034 (s, 1H, -NH), 8.610 (s, 1H, -CH); ^{13}C-NMR (δ (ppm) 163.4 (C=O, CONH), 157.2 (C-F, N=CH (C_6H_3FBr)),152.9 (C, C-4), 150.6 (C, C-3), 143.8 (CH, N=CH (C_6H_3FBr), 120.2 (C-Br, N=CH (C_6H_3FBr)), 126.8 (C, C-1), 122.9 (C, C-6), 114.8 (C, C-5), 115.6 (C, C-2), 56.5 (CH_3, OCH_3); Mass (m/z): 381 (M^+); Elemental analysis: Calculated: C, 50.41%; H, 3.70%; N; 7.35%; Found: C, 50.71%; H, 3.40%; N; 7.65%.

N'-(4-chlorobenzylidene)-3,4-dimethoxybenzohydrazide (V_{15}): Yield – 33.95%; Mp (°C) 138-140 ˚C; R_f value – 0.56; IR (cm^{-1}): 2937.70 (C-H str., -OCH_3), 3000.87 (C-H str., aromatic), 1599.09 (C=C str., aromatic), 716.97 (C-C out of plane bending, aromatic), 787.95 (C-H out of plane bending, aromatic), 896.37 (C-H deformed, aromatic), 1692.14 (C=N str.), 3251.79 (N-H str., 2° amide) 1658.97 (N-H in plane bending, 2° amide),1649.91(C=O str., 2°amide), 643.50 (C-Cl str.); ^{1}H-NMR (δ (ppm)): 3.403 (s, 6H, -OCH_3), 7.433-7.583(m, 4H, Ar-H), 7.730-7.899 (m, 3H, Ar-H), 8.443 (s, 1H, -NH), 8.688 (s, 1H, -CH); ^{13}C-NMR 163.2 (C=O, CONH), 151.2 (C, C-4), 148.8 (C, C-3), 142.9 (CH, N=CH (C_6H_4Cl), 137.8 (C-Cl, N=CH (C_6H_4Cl)), 128.2 (C, C-1), 119.7 (C, C-6), 116.5 (C, C-5), 113.4 (C, C-2), 56.4 (CH_3, OCH_3); Mass (m/z): 319 (M^+); Elemental analysis: Calculated: C, 60.29%; H, 4.74%; N, 8.79%; Found: C, 60.69%; H, 4.34%; N; 8.7%.

N'-(2-hydroxybenzylidene)-3,4-dimethoxybenzohydrazide (V_{16}): Yield – 75.16%; Mp (°C) 88-90 ˚C; R_f value – 0.64; IR(cm^{-1}): 2906.65 (C-H str., -OCH_3), 3020.03 (C-H str., aromatic), 1551.91 (C=C str., aromatic), 675.06(C-C out of plane bending, aromatic), 752.24 (C-H out of plane bending, aromatic), 879.25 (C-H deformed, aromatic), 1677.72 (C=N str.), 3137.88` (N-H str.,2° amide), 1563.05 (N-H in plane bending, 2° amide), 1677.72 (C=O str., 2°amide), 1226.92 (O-H str.); ^{1}H-NMR (δ (ppm)): 3.499 (s, 12H, -OCH_3), 7.221-7.315 (m, 4H, Ar-H), 7.700-7.996 (m, 3H, Ar-H), 8.209 (s, 1H, -NH), 8.557 (s, 1H, -CH); ^{13}C-NMR (ppm)) 162.9 (C=O, CONH), 162.3 (C-OH, N=CH (C_6H_4OH)), 154.3 (C, C-4), 150.8 (C, C-3), 143.6 (CH, N=CH (C_6H_4OH), 127.5 (C, C-1), 120.7 (C, C-6), 117.2 (C, C-5), 111.9 (C, C-2), 56.6 (CH_3, OCH_3); Mass (m/z): 300 (M^+); Elemental analysis: Calculated: C, 63.99%; H, 5.37%; N, 9.33%; Found: C, 63.97%; H, 5.38%; N, 9.36%.

N'-(2,4-dimethoxybenzylidene)-3,4-dimethoxybenzohydrazide (V_{17}): Yield – 39.68%; Mp (°C) 109-110 ˚C; R_f value – 0.46; IR(cm^{-1}): 2902.98 (C-H str., -OCH_3), 3040.15 (C-H str., aromatic), 1513.81 (C=C str., aromatic), 668.06 (C-C out of plane bending, aromatic), 768.21 (C-H out of plane bending, aromatic), 878.17 (C-H deformed, aromatic), 1677.75 (C=N str.), 3109.44 (N-H str., 2° amide), 1604.97 (N-H in plane bending, 2° amide), 1640.33 (C=O str., 2°amide); ^{1}H-NMR (δ (ppm)): 3.219 (s, 12H, -OCH_3), 7.115-7.231 (m, 3H, Ar-H), 7.378-7.454 (m, 3H, Ar-H), 8.334 (s, 1H, -NH), 8.561 (s, 1H, -CH); ^{13}C-NMR (ppm)) 163.7 (C=O, CONH), 153.2 (C, C-4), 146.9 (C, C-3), 143.6 (CH, N=CH (C_6H_3 (OCH_3)$_2$), 125.9 (C, C-1), 122.8 (C, C-6), 114.6 (C, C-5), 113.8 (C, C-2), 55.9 (CH_3, OCH_3); Mass (m/z): 344 (M^+); Elemental analysis: Calculated: C, 62.78%; H, 5.85%; N, 8.13%; Found: C, 62.79%; H, 5.82%; N, 8.15%.

N'-ethylidene-3,4-dimethoxybenzohydrazide (V_{18}):

Yield – 79.3%; Mp (°C) 168-170 ˚C; R_f value – 0.44; IR(cm^{-1}): 2945.32 (C-H str., -OCH_3), 2900.76 (C-H str., aliphatic), 1446.35 (C-H bending, aliphatic), 3024.56 (C-H str., aromatic), 1516.85 (C=C str., aromatic), 688.55 (C-C out of plane bending, aromatic), 758.82 (C-H out of plane bending, aromatic), 875.72 (C-H deformed, aromatic), 1695.44 (C=N str.), 3215.67 (N-H str., 2° amide), 1600.68 (N-H in plane bending, 2° amide),1646.99 (C=O str., 2°amide); ^{1}H-NMR (δ (ppm)): 3.406 (s, 6H, -OCH_3), 7.233-7.544 (m, 3H, Ar-H), 8.166 (s, 1H, -NH), 8.459 (s, 1H, -CH); ^{13}C-NMR (ppm)) 163.6 (C=O, CONH), 154.5 (CH, N=CH-CH_3), 151.8 (C, C-4), 149.9 (C, C-3), 128.9 (C, C-1), 118.9 (C, C-6), 117.1 (C, C-5), 111.5 (C, C-2), 56.9 (CH_3, OCH_3), 14.5 (CH_3, N=CH-CH_3); Mass (m/z): 222 (M^+); Elemental analysis: Calculated: C, 59.45%; H, 6.35%; N, 12.60% ; Found: C, 59.48%; H, 6.33%; N, 12.64%.

N'-(3-cyanobenzylidene)-3,4-dimethoxybenzohydrazide (V_{19}): Yield – 41.17%; Mp (°C) 198-200 ˚C; R_f value – 0.66; IR (cm^{-1}): 2967.97 (C-H str., -OCH_3), 3040.31 (C-H str., aromatic), 1530.12 (C=C str., aromatic), 699.90 (C-C out of plane bending, aromatic), 787.27 (C-H out of plane bending ,aromatic), 842.21 (C-H deformed, aromatic), 1677.67 (C=N str.), 3097.48 (N-H str., 2° amide), 1708.79 (N-H in plane bending, 2° amide),1649.47 (C=O str., 2°amide), 2357.28 (CN str. of aryl nitrile); ^{1}H-NMR (δ (ppm)): 3.726 (s, 6H, -OCH_3), 6.844-7.081 (m, 3H, Ar-H), 7.768-7.963 (m, 4H, Ar-H), 8.524 (s, 1H, -NH), 8.864 (s, 1H, -CH); ^{13}C-NMR (ppm)) 161.8 (C=O, CONH), 154.7 (C, C-4), 150.2 (C, C-3), 144.6 (CH, N=CH (C_6H_4CN)), 125.7 (C, C-1), 123.4 (C, C-6), 116.8 (C, C-5), 113.5 (C, C-2), 56.1 (CH_3, OCH_3), 116.4 (CN, N=CH (C_6H_4CN)). Mass (m/z): 309 (M^+); Elemental analysis: Calculated: C, 66.01%; H, 4.89%; N, 13.58%; Found: C, 66.03%; H, 4.87%; N, 13.56%.

N'-(4-formylbenzylidene)-3,4-dimethoxybenzohydrazide (V_{20}): Yield – 33.33%; Mp (°C) 188-190 ˚C; R_f value – 0.68; IR (cm^{-1}): 2974.81 (C-H str., -OCH_3), 3037.42 (C-H str., aromatic), 1537.39 (C=C str., aromatic), 631.24 (C-C out of plane bending, aromatic), 761.85 (C-H out of plane bending,

aromatic), 865.47 (C-H deformed, aromatic), 1646.36 (C=N str.), 3217.19 (N-H str., 2° amide), 1702.96 (N-H in plane bending, 2° amide), 1646.36 (C=O str., 2°amide), 1720.96 (C=O of aldehyde); ^{1}H-NMR (δ (ppm)): 3.551 (s, 6H, -OCH_3), 7.003-7.213 (m, 3H, Ar-H), 7.246-7.377 (m, 4H, Ar-H), 8.265 (s, 1H, -NH), 8.849 (s, 1H, -CH); ^{13}C-NMR (ppm)) 192 (C=O, N=CH (C_6H_4CO)), 162.5 (C=O, CONH), 153.1 (C, C-4), 149.2 (C, C-3), 143.0 (CH, N=CH (C_6H_4CO)), 128.6 (C, C-1), 120.8 (C, C-6), 113.4 (C, C-5), 110.5 (C, C-2), 55.7 (CH_3, OCH_3); Mass (m/z): 312 (M^+); Elemental analysis: Calculated: C, 65.38%; H, 5.16%; N, 8.97%; Found: C, 65.78%; H, 5.36%; N, 8.57%.

N'-(4-fluorobenzylidene)-3,4-dimethoxybenzohydrazide (V_{21}): Yield – 53.61%; Mp (°C) 109-110 ˚C; R_f value – 0.46; IR (cm^{-1}): 2975.69 (C-H str., -OCH_3), 3015.23 (C-H str., aromatic), 1511.17 (C=C str., aromatic), 691.24 (C-C out of plane bending, aromatic), 760.27 (C-H out of plane bending, aromatic), 869.33 (C-H deformed, aromatic), 1620.26 (C=N str.), 3189.42 (N-H str., 2° amide), 1720.48 (N-H in plane bending, 2° amide), 1665.46 (C=O str., 2°amide), 1323.26 (C-F str.); ^{1}H-NMR (δ (ppm)): 3.364 (s, 6H, -OCH_3), 7.038-7.172 (m, 4H, Ar-H), 7.265-7.494 (m, 3H, Ar-H), 8.460 (s, 1H, -NH), 8.704 (s, 1H, -CH); ^{13}C-NMR (ppm)) 165.2 (C-F, N=CH (C_6H_4F)), 163.4(C=O, CONH), 154.1 (C, C-4), 149.7 (C, C-3), 143.0 (CH, N=CH (C_6H_4F)), 126.3 (C, C-1), 122.4 (C, C-6), 117.3 (C, C-5), 113.8 (C, C-2), 56.8 (CH_3, OCH_3); Mass (m/z): 302 (M^+); Elemental analysis: Calculated: C, 63.57%; H, 5.00%; N, 9.27%; Found: C, 63.17%; H, 5.30%; N, 9.67%.

N'-(2-(p-tolylamino)acetyl)-3,4-dimethoxybenzohydrazide (V_{22}) IR(cm^{-1}): Yield – 49.27%; Mp (°C) 50-52˚C; R_f value – 0.33; 2906.28 (C-H str., -OCH_3), 2878.80 (C-H str., aliphatic), 1461.93 (C-H bending, aliphatic), 3000.38 (C-H str., aromatic), 1518.64 (C=C str., aromatic), 627.51 (C-C out of plane bending, aromatic), 787.75 (C-H out of plane bending , aromatic), 830.02 (C-H deformed, aromatic), 3140.79 (N-H str., 2° amide), 1631.02 (N-H in plane bending, 2° amide), 3491.13 (N-H str., 2°amine), 1587.11 (N-H bending, 2° amine), 1659.06 (C=O str., 2°amide); ^{1}H-NMR (δ (ppm)): 3.568 (s, 6H, -OCH_3), 3.783 (d, 2H, -CH_2), 4.225-4.295 (m, 3H, Ar-H), 7.239-7.600 (m, 5H, Ar-H), 8.301 (d, 1H, -NH of –CONHNH, J=7.1); ^{13}C-NMR (ppm)) 170.3 (C=O, CH_2-CONH), 164.9 (C=O, CONH), 151.6 (C, C-4), 149.8 (C, C-3), 129.2 (C, C-1), 119.7 (C, C-6), 114.5 (C, C-5), 112.4 (C, C-2), 58.9 (CH_2, CH_2CONH), 56.4 (CH_3, OCH_3), 24.3 (CH_3, NH ($C_6H_4CH_3$)); Mass (m/z): 343 (M^+); Elemental analysis: Calculated: C, 62.96%; H, 6.16%; N, 12.24%; Found: C, 63.12%; H, 6.09%; N, 12.04%.

N'-(2-(4-bromophenylamino)acetyl)-3,4-dimethoxybenzohydrazide (V_{23}): Yield – 90.55%; Mp (°C) 188-190 ˚C; R_f value – 0.34; IR(cm^{-1}): 2978.17 (C-H str., -OCH_3), 2936.98 (C-H str., aliphatic), 1488.70 (C-H bending, aliphatic), 3085.70 (C-H str., aromatic), 1547.30 (C=C str., aromatic), 691.90 (C-C out of plane bending, aromatic), 726.09 (C-H out of plane bending , aromatic), 876.03 (C-H deformed, aromatic), 3206.56 (N-H str., 2° amide), 1620.68 (N-H in plane bending, 2° amide), 3371.63 (N-H str., 2°amine), 1594.21 (N-H bending, 2° amine), 1639.23 (C=O str., 2°amide), 631.13 (C-Br str.); ^{1}H-NMR (δ (ppm)): 3.524 (s, 6H, -OCH_3), 2.560 (d, 2H, -CH_2), 7.105-7.344 (m, 4H, Ar-H), 7.365-7.748 (m, 3H, Ar-H), 8.011 (d, 1H, -NH of –CONHNH, J=7.3); ^{13}C-NMR (ppm)) 169.3 (C=O, CH_2-CONH),165.2 (C=O, CONH), 153.6 (C, C-4), 147.8 (C, C-3), 127.5 (C, C-1), 120.7 (C, C-6), 117.0 (C, C-5), 112.7 (C, C-2), 110.2 (C-Br, NH(C_6H_4Br)), 57.2 (CH_3, OCH_3), 56.5 (CH_2, CH_2CONH); Mass (m/z): 408 (M^+); Elemental analysis: Calculated: C, 50.01%; H, 4.44%; N, 10.29%; Found: C, 50.31%; H, 4.24%; N, 10.49%.

N'-(2-(4-methoxyphenylamino)acetyl)-3,4dimethoxybenzo hydrazide (V_{24}) Yield – 97.45%; Mp (°C) 148-150 ˚C; R_f value – 0.33; IR(cm^{-1}): 2868.07 (C-H str., -OCH_3), 2947.96 (C-H str., aliphatic), 1483.44 (C-H bending, aliphatic), 3000.27 (C-H str., aromatic), 1566.77 (C=C str., aromatic), 701.05 (C-C out of plane bending, aromatic), 721.42 (C-H out of plane bending, aromatic), 874.67 (C-H deformed, aromatic), 3163.49 (N-H str., 2° amide), 1692.10 (N-H in plane bending, 2° amide), 3525.40 (N-H str., 2°amine), 1566.94 (N-H bending, 2° amine), 1649.89 (C=O str., 2°amide); ^{1}H-NMR (δ (ppm)): 3.832 (s, 9H, -OCH_3), 2.536 (d, 2H, -CH_2, J=6.8), 7.517-7.581 (m, 4H, Ar-H), 7.000-7.027 (m, 3H, Ar-H), 8.231 (d, 1H, -NH of –CONHNH, J=7.0); ^{13}C-NMR (ppm)) 168.2 (C=O, CH_2-CONH), 163.2 (C=O, CONH), 151.4 (C, C-4), 149.9 (C, C-3), 126.4 (C, C-1), 118.7 (C, C-6), 114.9 (C, C-5), 112.7 (C, C-2), 58.4 (CH_2, CH_2CONH), 56.1 (CH_3, OCH_3); Mass (m/z): 359 (M^+); Elemental analysis: Calculated: C, 60.16%; H, 5.89%; N, 11.69%; Found: C, 60.36%; H, 5.49%; N, 11.29%.

3,4-dimethoxy-N'-(2-(phenylamino)acetyl)benzohydrazide (V_{25}): Yield – 92.59.%; Mp (°C) 110-119˚C; R_f value – 0.23; IR(cm^{-1}): 2906.87 (C-H str., -OCH_3), 2959.88 (C-H str., aliphatic), 1461.94 (C-H bending, aliphatic), 3077.03 (C-H str., aromatic), 1529.47 (C=C str., aromatic), 737.79 (C-C out of plane bending, aromatic), 763.87 (C-H out of plane bending ,aromatic), 873.67 (C-H deformed, aromatic), 3345.79 (N-H str., 2° amide), 1632.55 (N-H in plane bending, 2° amide), 3394.67 (N-H str., 2°amine), 1566.81 (N-H bending, 2° amine), 1673.20 (C=O str., 2°amide); ^{1}H-NMR (δ (ppm)): 3.239 (s, 6H, -OCH_3), 3.987 (d, 2H, -CH_2, J=6.9), 6.210-6.655 (m, 3H, Ar-H), 7.009-7.324 (m, 5H, Ar-H) 8.436 (d, 1H, -NH of –CONHNH, J=7.3); ^{13}C-NMR (ppm)) 171.1 (C=O, CH_2- CONH), 164.2 (C=O, CONH), 154.2 (C, C-4), 150.1 (C, C-3), 127.4 (C, C-1), 122.7 (C, C-6), 113.9 (C, C-5), 112.7 (C, C-2), 56.8 (CH_2, CH_2CONH), 56.6 (CH_3, OCH_3);Mass (m/z): 329 (M^+); Elemental analysis: Calculated: C, 62.00%; H, 5.81%; N, 12.76%; Found: C, 62.30%; H, 5.41%; N, 12.36%.

N'-(2-(4-chlorophenylamino)acetyl)-3,4-dimethoxy benzohydrazide (V_{26}): Yield – 45.55%; Mp (°C) 38-40 ˚C; R_f value – 0.48; IR(cm^{-1}): 2906.30 (C-H str., -OCH_3), 2868.15 (C-H str., aliphatic), 1493.18 (C-H bending, aliphatic), 3026.80 (C-H str., aromatic), 1530.10 (C=C str., aromatic), 691.43 (C-C out of plane bending, aromatic), 778.29 (C-H out of plane bending, aromatic), 894.02 (C-H deformed, aromatic), 3120.80 (N-H str.,2° amide), 1677.73 (N-H in plane bending, 2° amide), 3498.41 (N-H str., 2°amine), 1562.94 (N-H bending, 2° amine), 1649.87 (C=O str., 2°amide), 734.60 (C-Cl str.); ^{1}H-NMR (δ (ppm)): 3.734(s, 6H, -OCH_3), 2.879 (d, 2H, -CH_2, J=7.0), 6.885-7.295 (m, 4H, Ar-H), 7.390-7.608 (m, 3H, Ar-H), 8.650 (d, 1H, -NH of –CONHNH, J=7.2); ^{13}C-NMR (ppm)) 170.2 (C=O, CH_2- CONH), 164.9 (C=O, CONH), 155.4 (C, C-4), 147.1 (C, C-3), 129.8 (C, C-1), 122.8

(C-Cl, NH(C_6H_4Cl)), 120.7 (C, C-6), 115.1 (C, C-5), 114.7 (C, C-2), 57.4 (CH_2, CH_2CONH), 55.9 (CH_3, OCH_3); Mass (m/z): 364 (M^+); Elemental analysis: Calculated: C, 56.13%; H, 4.99%; N, 11.55%; Found: C, 56.33%; H, 4.79%; N, 11.35%.

N'-(2-(4-nitrophenylamino)acetyl)-3,4-dimethoxybenzohydrazide (V_{27}): Yield – 80.48%; Mp (°C) 98-100 ˚C; R_f value – 0.22; IR(cm^{-1}): 2948.14 (C-H str., -OCH_3), 2938.53 (C-H str., aliphatic), 1493.24 (C-H bending, aliphatic), 3044.05(C-H str., aromatic), 1547.53 (C=C str., aromatic), 681.20 (C-C out of plane bending, aromatic), 787.76 (C-H out of plane bending, aromatic), 895.89 (C-H deformed, aromatic), 3363.66 (N-H str., 2° amide), 1659.02 (N-H in plane bending, 2° amide), 3498.43 (N-H str., 2°amine), 1572.59 (N-H bending, 2° amine), 1659.02 (C=O str., 2°amide), 1352.00 (Ar-NO_2 str.); ^{1}H-NMR (δ (ppm)): 3.008 (s, 6H, -OCH_3), 2.784 (d, 2H, -CH_2, J=6.8), 7.235-7.315 (m, 3H, Ar-H), 7.366-7.748 (m, 4H, Ar-H), 8.883 (d, 1H, -NH of –CONHNH, J=7.5); ^{13}C-NMR (ppm)) 170.3 (C=O, CH_2- CONH), 164.9 (C=O, CONH), 151.6 (C, C-4), 149.8 (C, C-3), 136.8 (C-NO_2, NH($C_6H_4NO_2$)), 129.2 (C, C-1), 119.7 (C, C-6), 114.5 (C, C-5), 112.4 (C, C-2), 58.9 (CH_2, CH_2CONH), 56.4 (CH_3, OCH_3); Mass (m/z): 374 (M^+); Elemental analysis: Calculated: C, 54.54%; H, 4.85%; N, 14.97%; Found: C, 54.24%; H, 4.55%; N, 14.67%.

N'-(2-(2-chlorophenylamino)acetyl)-3,4-dimethoxybenzohydrazide (V_{28}): Yield – 95.83%; Mp (°C) 158-160 ˚C; R_f value – 0.60; IR(cm^{-1}): 2859.83 (C-H str., -OCH_3), 2924.34 (C-H str., aliphatic), 1484.18 (C-H bending, aliphatic), 3075.70 (C-H str., aromatic), 1552.18 (C=C str., aromatic), 668.11 (C-C out of plane bending, aromatic), 751.50 (C-H out of plane bending, aromatic), 873.21 (C-H deformed, aromatic), 3154.25 (N-H str., 2° amide), 1632.72 (N-H in plane bending, 2° amide), 3515.49 (N-H str., 2°amine), 1585.06 (N-H bending, 2° amine), 1673.14 (C=O str., 2°amide), 621.75 (C-Cl str.); ^{1}H-NMR (δ (ppm)): 3.562 (s, 6H, -OCH_3,), 3.812 (d, 2H, -CH_2, J=6.4), 7.012-7.277 (m, 3H, Ar-H), 7.318-7.604 (m, 4H, Ar-H), 7.566 (d, 1H, -NH of –CONHNH, J=7.1); ^{13}C-NMR (ppm)) 169.3 (C=O, CH_2-CONH),165.2 (C=O, CONH), 153.6 (C, C-4), 147.8 (C, C-3), 127.5 (C, C-1), 123.5 (C-Cl, NH(C_6H_4Cl)), 120.7 (C, C-6), 117.0 (C, C-5), 112.7 (C, C-2), 57.2 (CH_3, OCH_3), 56.5 (CH_2, CH_2CONH); Mass (m/z): 364 (M^+); Elemental analysis: Calculated: C, 56.13%; H, 4.99%;N, 11.55%; Found: C, 56.33%; H, 4.59%; N, 11.35%.

N'-(2-(3-nitrophenylamino)acetyl)-3,4-dimethoxybenzohydrazide (V_{29}): Yield – 67.74%; Mp (°C) 188-190 ˚C; R_f value – 0.26; IR(cm^{-1}): 2961.64 (C-H str., -OCH_3), 2937.55 (C-H str., aliphatic), 1416.73 (C-H bending, aliphatic), 3088.78 (C-H str., aromatic), 1525.41 (C=C str., aromatic), 673.70 (C-C out of plane bending, aromatic), 759.91 (C-H out of plane bending, aromatic), 874.08 (C-H deformed, aromatic), 3374.04 (N-H str., 2° amide), 1632.91 (N-H in plane bending, 2° amide), 3469.53 (N-H str., 2°amine), 1599.13 (N-H bending, 2° amine), 1632.91 (C=O str., 2°amide), 1349.46 (Ar-NO_2 str.); ^{1}H-NMR (δ (ppm)): 3.880(s, 6H, -OCH_3), 2.985 (d, 2H, -CH_2, J=6.9), 7.334-7.421 (m, 3H, Ar-H), 7.546-7.684 (m, 3H, Ar-H), 8.264 (d, 1H, -NH of –CONHNH, J=7.5); ^{13}C-NMR (ppm)) 169.9 (C=O, CH_2-CONH), 163.2 (C=O, CONH), 151.4 (C, C-4), 150.2 (C-NO_2, NH ($C_6H_4NO_2$)), 149.9 (C, C-3), 126.4 (C, C-1), 118.7 (C, C-6), 114.9 (C, C-5), 112.7 (C, C-2), 58.4 (CH_2, CH_2CONH), 56.1 (CH_3, OCH_3); Mass (m/z): 409 (M^+); Elemental analysis: Calculated: C, 49.95%; H, 4.19%; N, 13.71%; Found: C, 49.55%; H, 4.39%; N, 13.31%.

N'-(2-(4-chloro-2-nitrophenylamino)acetyl)-3,4-dimethoxybenzohydrazide (V_{30}): Yield – 35.06%; Mp (°C) 76-78 ˚C; R_f value – 0.41; IR(cm^{-1}): 2983.38 (C-H str., -OCH_3), 2928.20 (C-H str., aliphatic), 1485.52 (C-H bending, aliphatic), 3024.55 (C-H str., aromatic), 1548.03 (C=C str., aromatic), 665.47(C-C out of plane bending, aromatic), 791.91 (C-H out of plane bending, aromatic), 896.91 (C-H deformed, aromatic), 3126.77 (N-H str., 2° amide), 1677.08 (N-H in plane bending, 2° amide), 3457.16 (N-H str., 2°amine), 1585.51 (N-H bending, 2° amine), 1641.01 (C=O str., 2°amide), 1371.99(Ar-NO_2 str.), 752.16 (C-Cl str.); ^{1}H-NMR (δ (ppm)): 3.320 (s, 6H, -OCH_3), 2.151 (d, 2H, -CH_2, J=6.7), 7.1.34-7.421 (m, 3H, Ar-H ,J=86.1), 7.661-7.784 (m, 3H, Ar-H) 8.044 (d, 1H, -NH of –CONHNH, J=7.0); ^{13}C-NMR (ppm)) 170.2 (C=O, CH_2- CONH), 164.9 (C=O, CONH), 155.4 (C, C-4), 147.1 (C, C-3), 133.5 (C-NO_2, NH($C_6H_4ClNO_2$)), 129.8 (C, C-1), 123.8 (C-Cl, NH($C_6H_4ClNO_2$)), 120.7 (C, C-6), 115.1 (C, C-5), 112.3 (C, C-2), 57.4 (CH_2, CH_2CONH), 55.9 (CH_3, OCH_3); Mass (m/z): 409 (M^+); Elemental analysis: Calculated: C, 49.95%; H, 4.19%; N, 13.71%; Found: C, 49.55%; H, 4.49%; N, 13.31%.

N'-(2-(3-chlorophenylamino)acetyl)-3,4-dimethoxybenzohydrazide (V_{31}): Yield – 98.33%; Mp (°C) 178-180 ˚C; R_f value – 0.50; IR(cm^{-1}): 2838.91 (C-H str., -OCH_3), 2937.20 (C-H str., aliphatic), 1463.45 (C-H bending, aliphatic), 3054.48 (C-H str., aromatic), 1511.57 (C=C str., aromatic), 680.68 (C-C out of plane bending, aromatic), 760.53 (C-H out of plane bending, aromatic), 885.92 (C-H deformed, aromatic), 3230.12 (N-H str., 2° amide), 1596.20 (N-H in plane bending, 2° amide), 3469.10 (N-H str., 2°amine), 1511.57 (N-H bending, 2° amine), 1698.77 (C=O str., 2°amide), 630.99 (C-Cl str.); ^{1}H-NMR (δ (ppm)): 3.265 (s, 6H,-OCH_3), 3.763 (d, 2H, -CH_2, J=7.1), 7.199-7.248 (m, 3H, Ar-H), 6.958-7.080 (m, 4H, Ar-H), 7.561 (d, 1H, -NH of –CONH, J=8.2); ^{13}C-NMR (ppm)) 170.2 (C=O, CH_2- CONH), 166.9 (C=O, CONH), 155.4 (C, C-4), 147.1 (C, C-3), 136.4 (C-Cl, NH(C_6H_4Cl)), 129.8 (C, C-1), 120.7 (C, C-6), 115.1 (C, C-5), 114.7 (C, C-2), 57.4 (CH_2, CH_2CONH), 56.9 (CH_3, OCH_3); Mass (m/z): 364 (M^+); Elemental analysis: Calculated: C, 56.13%; H, 4.99%; N, 11.55%; Found: C, 56.53%; H, 4.89%; N, 11.25%.

N'-(2-(2,4-dimethylphenylamino)acetyl)-3,4-dimethoxybenzohydrazide (V_{32}): Yield – 76.92%; Mp (°C) 188-190 ˚C; R_f value – 0.40; IR(cm^{-1}): 2976.91 (C-H str., -OCH_3), 2920.54 (C-H str., aliphatic), 1494.05 (C-H bending, aliphatic), 3034.08 (C-H str., aromatic), 1560.74 (C=C str., aromatic), 698.04 (C-C out of plane bending, aromatic),753.30 (C-H out of plane bending ,aromatic), 869.27 (C-H deformed, aromatic), 3180.21 (N-H str., 2° amide), 1634.65 (N-H in plane bending, 2° amide), 3498.32 (N-H str., 2°amine), 1585.00 (N-H bending, 2° amine), 1645.80 (C=O str., 2°amide), 764.87 (C-Cl str.); ^{1}H-NMR (δ (ppm)): 3.541 (s, 6H, -OCH_3), 3.623 (d, 2H, -CH_2, J=6.5), 7.055-7.281 (m, 3H, Ar-H), 6.658-6.784 (m, 3H, Ar-H), 8.599 (d, 1H, -NH of –

CONHNH, J=7.2); ^{13}C-NMR (ppm)) 169.3 (C=O, CH_2-CONH), 162.4 (C=O, CONH), 154.4 (C, C-4), 149.9 (C, C-3), 126.4 (C, C-1), 117.7 (C, C-6), 113.9 (C, C-5), 111.7 (C, C-2), 58.4 (CH_2, CH_2CONH), 56.1 (CH_3, OCH_3), 24.3 (CH_3, $NH(C_6H_4(CH_3)_2)$; Mass (m/z): 358 (M^+); Elemental analysis: Calculated: C, 63.85%; H, 6.49%; N, 11.76%; Found: C, 63.45%; H, 6.89% ; N, 11.56%.

N'-(2-(m-tolylamino)acetyl)-3,4-dimethoxybenzohydrazide (V_{33}): Yield – 70.79%; Mp (°C) 112-114 °C; R_f value – 0.21; IR(cm^{-1}): 2963.78 (C-H str., -OCH_3), 2920.99 (C-H str., aliphatic), 1492.41 (C-H bending, aliphatic), 3009.39 (C-H str., aromatic), 1513.83 (C=C str., aromatic), 644.87 (C-C out of plane bending, aromatic), 728.06 (C-H out of plane bending , aromatic), 889.38 (C-H deformed, aromatic), 3355.73 (N-H str., 2° amide), 1643.61 (N-H in plane bending, 2° amide), 3485.34 (N-H str., 2°amine), 1618.95 (N-H bending, 2° amine), 1650.80 (C=O str., 2°amide); ^{1}H-NMR (δ (ppm)): 3.331 (s, 6H, -OCH_3), 3.654 (d, 2H, -CH_2, J=6.9), 7.258-7.380 (m, 3H, Ar-H), 6.624-6.738 (m, 4H, Ar-H), 8.677 (d, 1H, -NH of –CONHNH, J=7.2); ^{13}C-NMR (ppm)) 172.2 (C=O, CH_2-CONH), 164.9 (C=O, CONH), 155.4 (C, C-4), 150.0 (C, C-3), 129.8 (C, C-1), 120.2 (C, C-6), 116.1 (C, C-5), 110.7 (C, C-2), 57.6 (CH_2, CH_2CONH), 55.9 (CH_3, OCH_3), 24.9 (CH_3, $NH(C_6H_4(CH_3)_2)$; Mass (m/z): 343 (M^+); Elemental analysis: Calculated: C, 62.96%; H, 6.16%; N, 12.24%; Found: C, 62.56%; H, 6.36% ; N, 12.44%.

N'-(2-(2-nitrophenylamino)acetyl)-3,4-dimethoxybenzohydrazide (V_{34}): Yield – 87.80%; Mp (°C) 164-168 °C; R_f value – 0.40; IR(cm^{-1}): 2852.26 (C-H str., -OCH_3), 2953.72 (C-H str., aliphatic), 1485.45 (C-H bending aliphatic), 3037.88 (C-H str., aromatic), 1536.32 (C=C str., aromatic), 723.02 (C-C out of plane bending, aromatic), 740.52 (C-H out of plane bending, aromatic), 898.47 (C-H deformed, aromatic), 3360.25 (N-H str., 2° amide), 1673.36 (N-H in plane bending, 2° amide), 3485.10 (N-H str., 2°amine, 1626.28 (N-H bending, 2° amine), 1673.36 (C=O str., 2°amide), 1391.95,1536.32 (Ar-NO_2 str.); ^{1}H-NMR (δ (ppm)): 3.572 (s, 6H, -OCH_3), 3.816 (d, 2H, -CH_2, J=6.8), 6.573 -7.092 (m, 3H, Ar-H), 7.224 -7.604 (m, 4H, Ar-H), 7.946 (d, 1H, -NH of –CONH J=8.0); ^{13}C-NMR (ppm)) 169.9 (C=O, CH_2- CONH), 165.1 (C=O, CONH), 152.9 (C, C-4), 149.8 (C, C-3), 132.5 (C-NO_2, $NH(C_6H_4NO_2)$), 127.5 (C, C-1), 120.8 (C, C-6), 112.8 (C, C-5), 111.6 (C, C-2), 57.3 (CH_2, CH_2CONH), 56.2 (CH_3, OCH_3); Mass (m/z): 374 (M^+); Elemental analysis: Calculated: C, 54.54%; H, 4.85%; N, 14.97%; Found: C, 54.84%; H, 4.45%; N, 14.47%.

Antimicrobial evaluation

Antibacterial assay

A 24 h fresh culture was obtained by inoculation of respective bacteria in nutrient broth I.P (Pharmacopoeia of India, 1996) followed by incubation at 37± 1°C. Test solution (1 ml, 100 µg/ml) was transferred in one tube and serially diluted to give a concentration of 50, 25, 12.5, 6.25, 3.12 µg/ml. To all the tubes 0.1 ml of suspension of respective microorganisms (Gram-positive *S. aureus MTCC 2901*, *B. subtilis MTCC 2063*, Gram-negative *E. coli MTCC 1652*) in normal saline was added as inoculum and the 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. The procedure was performed for three bacterial species for 34 test compounds and activity was compared with standard (ciprofloxacin). b

Antifungal assay

The antifungal activity of synthesized veratric acid derivatives against the fungal species (*C. albicans MTCC 227* and *A.niger MTCC 8189*) were performed by standard serial dilution method (Park *et al.*, 2004) (similar to antibacterial assay mentioned above) by use of Sabouraud's dextrose broth IP as media for assay. 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. The activity of the compounds was compared with the standard (fluconazole).

QSAR studies

Data set is the set of molecules whose biological activity is regressed with its molecular descriptor values. Our data set consisted of 34 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 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 (TSAR 3D Version 3.3, 2000). Further, the regression analysis was performed using the SPSS software package (SPSS for Windows, 1999**).**

Cross-validation

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

$$q^2 = 1 - \sum (Y_{predicted} - Y_{actual})^2 / \sum (Yactual - Ymean)^2$$

where $Y_{predicted}$, Y_{actual} and Y_{mean} are the predicted, actual and mean values of target property (pMIC), respectively.

$\sum (Y_{predicted} - Y_{actual})^2$ is the predictive residual error sum of squares.

Results and Discussion

Synthesis of veratric acid derivatives (V_1 - V_{34})

Synthetic route to compounds (V_1 -V_{21}) and (V_{22}–V_{34}) is shown in Scheme I and II, respectively. In first series, veratric acid was treated with ethanol in the presence of mineral acid and ethyl veratrate was formed. Then the reaction was carried out between ethyl veratrate and hydrazine hydrate which resulted

into the formation of veratric acid hydrazide. Finally veratric acid hydrazide reacted with different aldehydes to form various schiff base derivatives (V_1 -V_{21}) (Scheme 1). In second series, the reaction of veratric acid hydrazide with chloro acetyl chloride was carried out which furnished chlorinated acetylated derivatives. The yielded compounds then reacted with corresponding anilines to form benzohydrazide derivatives (V_{22}–V_{34}) (Scheme 2). All the compounds were obtained in appreciable yield. The formation of target compounds was ascertained on the basis of results of elemental analysis in addition to their consistent IR and NMR spectral characteristics. The results of elemental analysis of synthesized compounds were in all case within 0.4% of theoretical values and were in confirmation of desired structure.

The most prominent and most informative bands in the spectra of aromatic compounds occur in the low-frequency range between 898-627cm^{-1}. The presence of peaks slightly above and below 3000 cm^{-1} indicated the presence of an aromatic and aliphatic portion in the synthesized compounds, respectively. The skeletal C=C stretching bands (aromatic) were observed around 1599-1457 cm^{-1} in the spectra of the synthesized compounds which represents the presence of aromatic nucleus in their structure. Further C=N stretching band at 1695-1620 cm^{-1} indicated the formation of schiff bases and hence confirm the formation of target compounds. In IR spectra of secondary amides, which exist mainly in trans conformations, the free N-H stretching vibration observed in 3545-3097cm^{-1}. The C=O absorption of amides occurs in the range of 1698 -1632cm^{-1}. The N-H stretching and bending vibrations in secondary amines were observed in the range of 3556-3210 cm^{-1} and1602 -1511 cm^{-1} respectively. C-Cl str. in chlorinated compounds (V_{12}, V_{15}, V_{26}, V_{28}, V_{30}, V_{31}) was observed in the range of 757-621 cm^{-1}. Brominated compounds (V_{14}, V_{23}) absorbed in the 640-631 region corresponding to C-Br stretching. Fluorine containing compounds (V_{14}, V_{21}) absorbed strongly over a wide range between 1323- 1270 cm^{-1} because of C-F stretching modes.

The formation of schiff bases were confirmed by the appearance of singlet signal around δ 8.459-8.956 ppm. The appearance of multiplet signal around δ 7.006-7.996 ppm depicted the presence of aromatic protons. Singlet signal observed in the range δ 3.194-3.950 ppm indicated the presence of methoxy group. Doublet signal corresponding to range of δ 2.151-3.816 ppm confirmed the presence of methylene bridge. The singlet signals observed around δ 8 ppm and δ 9 ppm represents the NH protons at N_2 and N_1 position of the hydrazide portion in compounds (V_{22}–V_{34}). This clearly signifies that the acyl group was attached to the veratric acid hydrazide (V_{36}) by replacing one of the two protons of NH_2 group. The singlet signal for COOH proton of compound V_{38} was observed at 11.94 ppm and disappearance of this signal in all synthesized compounds confirmed their formation.

Antimicrobial activity

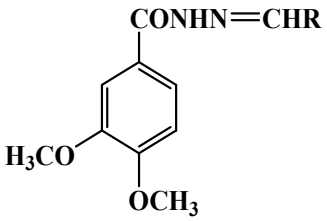
The results of antimicrobial study are presented in Table 1. The synthesized compounds (V_{15}, V_{31}) exhibited most promising antimicrobial activity against panel of microorganism *S. aureus, B. subtilis, E. coli, C. albicans, A. niger* (MIC=12.5 µg/ml). The title compounds (V_9, V_{24}) showed significant antimicrobial activity against *S. aureus* (MIC=6.25µg/ml). Compounds (V_{14}, V_{34}) showed pronounced activity against *B. subtilis* (MIC=6.25 µg/ml). Compounds (V_{14}, V_{28}) were found to have remarkable activity against *E.coli* (MIC=6.25 µg/ml). Compounds (V_2, V_{21}, V_{25}, V_{28}) demonstrated most significant activity against *C. albicans* (MIC=6.25 µg/ml). Compounds (V_1, V_{28}) exhibited marked activity against *A. niger* (MIC=6.25 µg/ml). The outcome of the study suggested that the test compound V_{28} may be utilized as potential antimicrobial agent against *S. aureus, B. subtilis, E. coli, C. albicans, A. niger*.

From the results of antimicrobial activity the following conclusions regarding structure activity relationship (SAR) can be drawn:

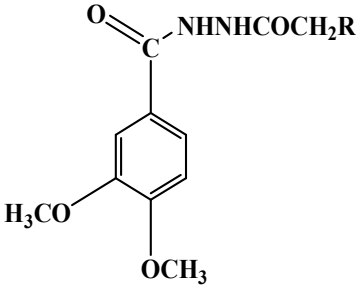
- The presence of electron withdrawing groups like Cl, NO_2, F and Br improved the antimicrobial activity of veratric acid derivatives which is evidenced by the antimicrobial data of compounds V_2, V_{14}, V_{15}, V_{28}, V_{31} and V_{34} in Table 1 against different representative bacteria. The role of electron withdrawing group in improving the antimicrobial activities is supported by the studies of Sharma et al., 2004.
- In general benzohydrazide derivatives of veratric acid have more antimicrobial activity than its schiff bases. The exceptionally high antimicrobial activity shown by compound V_{28}, V_{31}, V_{34} of veratric acid may be due to the presence of electron withdrawing group. Further, the amides with electron withdrawing group were generally more active which may be attributed to the presence of aromatic ring. The positive contribution of aromatic ring may be due to the involvement of aromatic ring in enhancing the binding of molecules with the target.
- Analysis of results indicated that the presence of an electron withdrawing NO_2 group (compounds V_2, V_{34}) leads to increase in activity in comparison to presence of other group.
- The presence of the chloro group at ortho position of phenyl portion of compound V_{28} as compared to chloro group at meta and para position in compound V_{26}, V_{31} increased antimicrobial activity against *B.subtilis*, *C. albicans* and *A. niger* with MIC= 6.25 µg/mL.
- Presence of electron withdrawing 2-floro-5-bromo substituents on phenyl portion (V_{14}) increased the antimicrobial activity against *B. subtilis* and *E. coli*.
- In contrast with Tripathi et al., 2006 who stated that the OH group at ortho position leads to a measurable change in activity of the compounds, the presence of the OH group at ortho position of naphthyl portion of compound V_7 did not improve antimicrobial activity of the compound.
- Among the different electron withdrawing groups, chloro and bromo groups were most effective in conferring the antimicrobial activity to potential.
- Presence of electron releasing group on phenyl portion (V_3, V_{10}, V_{13} and V_{17}) and on phenylimino portion of the synthesized compound (V_{24} and V_{33}) did not improve antimicrobial potential.

The aforementioned results indicated the fact that different structural requirements are essential for a compound to be selected as antibacterial or antifungal agent. This is similar to the results obtained by Sortino *et al*., 2007.

Table 1. Antimicrobial activities of veratric acid derivatives



Schiff bases



$V_1 - V_{21}$ $V_{22} - V_{34}$

Comp.	R	Molecular formula	S.A MIC(µg/ml)	E.C MIC(µg/ml)	B.S MIC(µg/ml)	C.A MIC(µg/ml)	A.N MIC(µg/ml)
V_1	-H	$C_{10}H_{12}N_2O_3$	25	25	25	12.5	6.25
V_2	$C_6H_4NO_2$	$C_{16}H_{15}N_3O_4$	25	25	25	6.25	12.5
V_3	$C_6H_4NO_2$	$C_{16}H_{15}N_3O_4$	25	25	25	12.5	12.5
V_4	$C_6H_4OCH_3$	$C_{17}H_{18}N_2O_4$	12.5	25	25	12.5	12.5
V_5	C_6H_5	$C_{16}H_{16}N_2O_3$	12.5	12.5	25	12.5	12.5
V_6	C_4H_3O	$C_{14}H_{14}N_2O_4$	25	12.5	25	12.5	12.5
V_7	$C_{10}H_6OH$	$C_{20}H_{18}N_2O_4$	12.5	25	12.5	12.5	12.5
V_8	$C_6H_5C_3H_5$	$C_{18}H_{18}N_2O_4$	25	25	25	12.5	12.5
V_9	$C_6H_3(OCH_3)_2$	$C_{18}H_{20}N_2O_5$	6.25	12.5	25	12.5	12.5
V_{10}	$C_6H_4OCH_3$	$C_{17}H_{18}N_2O_4$	25	12.5	12.5	12.5	12.5
V_{11}	$C_6H_4N(CH_3)_2$	$C_{18}H_{21}N_3O_3$	25	25	12.5	12.5	12.5
V_{12}	C_6H_4Cl	$C_{16}H_{15}ClN_2O_3$	25	25	25	12.5	12.5
V_{13}	$C_6H_4OCH_3$	$C_{17}H_{18}N_2O_4$	12.5	25	12.5	12.5	12.5
V_{14}	C_6H_3FBr	$C_{16}H_{14}BrFN_2O_3$	12.5	6.25	6.25	12.5	12.5
V_{15}	C_6H_4Cl	$C_{16}H_{15}ClN_2O_3$	12.5	12.5	12.5	12.5	12.5
V_{16}	C_6H_4OH	$C_{16}H_{16}N_2O_4$	25	25	25	12.5	12.5
V_{17}	$C_6H_3(OCH_3)_2$	$C_{18}H_{20}N_2O_5$	12.5	25	12.5	12.5	12.5
V_{18}	$-CH_3$	$C_{11}H_{14}N_2O_3$	25	12.5	25	12.5	12.5
V_{19}	C_6H_4CN	$C_{17}H_{15}N_3O_3$	25	12.5	25	12.5	12.5
V_{20}	C_6H_4CHO	$C_{17}H_{16}N_2O_4$	25	25	25	12.5	12.5
V_{21}	C_6H_4F	$C_{16}H_{15}FN_2O_3$	12.5	25	12.5	6.25	12.5
V_{22}	C_7H_8N	$C_{18}H_{21}N_3O_4$	12.5	25	25	12.5	12.5
V_{23}	C_6H_5BrN	$C_{17}H_{18}BrN_3O_4$	12.5	25	12.5	12.5	12.5
V_{24}	C_7H_8NO	$C_{18}H_{21}N_3O_5$	6.25	12.5	12.5	12.5	12.5
V_{25}	C_6H_6N	$C_{17}H_{19}N_3O_4$	12.5	25	12.5	6.25	12.5
V_{26}	C_6H_5ClN	$C_{17}H_{18}ClN_3O_4$	12.5	12.5	25	12.5	12.5
V_{27}	$C_6H_5N_2O_2$	$C_{17}H_{18}N_4O_6$	25	12.5	12.5	12.5	12.5
V_{28}	C_6H_5ClN	$C_{17}H_{18}ClN_3O_4$	12.5	6.25	12.5	6.25	6.25
V_{29}	$C_6H_5N_2O_2$	$C_{17}H_{17}ClN_4O_6$	25	25	25	12.5	12.5
V_{30}	$C_6H_4ClN_2O_2$	$C_{17}H_{17}ClN_4O_6$	25	25	25	12.5	12.5
V_{31}	C_6H_5ClN	$C_{17}H_{18}ClN_3O_4$	12.5	12.5	12.5	12.5	12.5
V_{32}	$C_8H_{10}N$	$C_{19}H_{23}N_3O_4$	25	12.5	12.5	12.5	12.5
V_{33}	C_7H_8N	$C_{18}H_{21}N_3O_4$	25	25	25	12.5	12.5
V_{34}	$C_6H_5N_2O_2$	$C_{17}H_{18}N_4O_6$	12.5	12.5	6.25	12.5	12.5

Quantitative structure activity relationship studies

Quantitative structure activity relationship (QSAR) is a predictive tool for preliminary evaluation of the activity of chemical compounds by using computer-aided models. In order to identify the substituent effect on the antimicrobial activity, QSAR studies were undertaken, using the linear free energy relationship (LFER) model described by Hansch and Fujita (Hansch *et al.,* 1964). Biological activity data determined as MIC values were first transformed into pMIC values (*i.e.* –log MIC) and used as dependent variables in QSAR study (Table 2). The different molecular descriptors selected for the present study are listed in Table 3 and values of selected molecular descriptors calculated for the synthesized compounds (V_1- V_{34}) are presented in Table 4.

In the present study, we attempted to develop three different types of *mt*-QSAR models *viz. mt*-QSAR model for describing antibacterial activity of synthesized compounds *against S. aureus, B. subtilis* and *E. coli*, *mt*-QSAR model for describing antifungal activity of synthesized compounds against *C. albicans* and *A. niger* as well as a common *mt*-QSAR model for describing the antimicrobial (overall antibacterial and antifungal) activity of synthesized compounds by calculating their average antibacterial activity, antifungal activity and antimicrobial activity values which are presented in Table 2.

Our previous studies in the field of QSAR (Kumar *et al*., 2012, Judge *et al*., 2012; Narang *et al*., 2012) indicated that the multi-target QSAR (*mt*-QSAR) models are better than one-target QSAR (*ot*-QSAR) models in describing the antimicrobial activity. So, in the present study we have developed multi-target QSAR models to describe the antimicrobial activity of synthesized 3, 4-dimethoxybenzoic acid derivatives. According to the *ot*-QSAR models, one should use five different equations with different errors to predict the activity of a new compound against five microbial species.

Table 2. Antimicrobial activity (pMIC in µmol/ml) of synthesized compounds

S.No.	$pMIC_{sa}$	$pMIC_{ec}$	$pMIC_{bs}$	$pMIC_{ca}$	$pMIC_{an}$	$pMIC_{ab}$	$pMIC_{af}$	$pMIC_{am}$
V_1	0.92	0.92	0.92	1.22	1.52	0.92	1.37	1.10
V_2	1.12	1.12	1.12	1.72	1.42	1.12	1.57	1.30
V_3	1.12	1.12	1.12	1.42	1.42	1.12	1.42	1.24
V_4	1.40	1.10	1.10	1.40	1.40	1.20	1.40	1.28
V_5	1.36	1.36	1.06	1.36	1.36	1.26	1.36	1.30
V_6	1.04	1.34	1.04	1.34	1.34	1.14	1.34	1.22
V_7	1.45	1.15	1.45	1.45	1.45	1.35	1.45	1.39
V_8	1.09	1.09	1.09	1.39	1.39	1.09	1.39	1.21
V_9	1.74	1.44	1.14	1.44	1.44	1.44	1.44	1.44
V_{10}	1.10	1.40	1.40	1.40	1.40	1.30	1.40	1.34
V_{11}	1.12	1.12	1.42	1.42	1.42	1.22	1.42	1.30
V_{12}	1.11	1.11	1.11	1.41	1.41	1.11	1.41	1.23
V_{13}	1.40	1.10	1.40	1.40	1.40	1.30	1.40	1.34
V_{14}	1.48	1.79	1.79	1.48	1.48	1.68	1.48	1.60
V_{15}	1.41	1.41	1.41	1.41	1.41	1.41	1.41	1.41
V_{16}	1.08	1.08	1.08	1.38	1.38	1.08	1.38	1.20
V_{17}	1.44	1.14	1.44	1.44	1.44	1.34	1.44	1.38
V_{18}	0.95	1.25	0.95	1.25	1.25	1.05	1.25	1.13
V_{19}	1.09	1.39	1.09	1.39	1.39	1.19	1.39	1.27
V_{20}	1.10	1.10	1.10	1.40	1.40	1.10	1.40	1.22
V_{21}	1.38	1.08	1.38	1.68	1.38	1.28	1.53	1.38
V_{22}	1.44	1.14	1.14	1.44	1.44	1.24	1.44	1.32
V_{23}	1.51	1.21	1.51	1.51	1.51	1.41	1.51	1.45
V_{24}	1.76	1.46	1.46	1.46	1.46	1.56	1.46	1.52
V_{25}	1.42	1.12	1.42	1.72	1.42	1.32	1.57	1.42
V_{26}	1.46	1.46	1.16	1.46	1.46	1.36	1.46	1.40
V_{27}	1.18	1.48	1.48	1.48	1.48	1.38	1.48	1.42
V_{28}	1.46	1.76	1.46	1.76	1.76	1.56	1.76	1.64
V_{29}	1.21	1.21	1.21	1.51	1.51	1.21	1.51	1.33
V_{30}	1.21	1.21	1.21	1.51	1.51	1.21	1.51	1.33
V_{31}	1.46	1.46	1.46	1.46	1.46	1.46	1.46	1.46
V_{32}	1.16	1.46	1.46	1.46	1.46	1.36	1.46	1.40
V_{33}	1.14	1.14	1.14	1.44	1.44	1.14	1.44	1.26
V_{34}	1.48	1.48	1.78	1.48	1.48	1.58	1.48	1.54
S.D.	0.21	0.20	0.22	0.12	0.08	0.17	0.09	0.12
Std.	2.61*	2.61*	2.61*	2.64**	2.64**	-	-	-

*Ciprofloxacin **Fluconazole

Table 3. QSAR descriptors used in the study

S. No.	QSAR descriptor	Type
1.	log P	Lipophilic
2.	Zero order molecular connectivity index ($^0\chi$)	Topological
3.	First order molecular connectivity index ($^1\chi$)	Topological
4.	Second order molecular connectivity index ($^2\chi$)	Topological
5.	Valence zero order molecular connectivity index ($^0\chi^v$)	Topological
6.	Valence first order molecular connectivity index ($^1\chi^v$)	Topological
7.	Valence second order molecular connectivity index ($^2\chi^v$)	Topological
8.	Kier's alpha first order shape index ($\kappa\alpha_1$)	Topological
9.	Kier's alpha second order shape index ($\kappa\alpha_2$)	Topological
10.	Kier's first order shape index (κ_1)	Topological
11.	Randic topological index	Topological
12.	Balaban topological index	Topological
13.	Wiener's topological index	Topological
14.	Kier's second order shape index (κ_2)	Topological
15.	Ionization potential	Electronic
16.	Dipole moment (μ)	Electronic
17.	Energy of highest occupied molecular orbital (HOMO)	Electronic
18.	Energy of lowest unoccupied molecular orbital (LUMO)	Electronic
19.	Total energy (Te)	Electronic
20.	Nuclear Energy (Nu. E)	Electronic
21.	Molar refractivity (MR)	Steric

The utilization of *ot*-QSAR models, which are almost in the whole literature however, were not practical when we had to predict each compound results for more than one target. In those cases we had to develop one *ot*-QSAR for each target. However, very recently the interest has been increased in the development of multi-target QSAR (*mt*- QSAR) models.

As opposed to *ot*-QSAR, the *mt*-QSAR model is a single equation that considers the nature of molecular descriptors which are common and essential for describing the antibacterial and antifungal activity (Gonzalez-Diaz *et al*., 2008; Cruz-Monteagudo *et al*., 2007; Gonzalez-Diaz *et al*., 2007 and Gonzalez-Diaz *et al*., 2008).

Table 4. Values of selected molecular descriptors used in QSAR study

S. No.	log P	MR	$^0\chi$	$^0\chi^v$	$\kappa\alpha_1$	$\kappa\alpha_2$	Te	LUMO	HOMO	μ
V_1	0.44	54.33	11.26	8.61	11.91	5.66	-2818.35	-0.39	-9.01	4.89
V_2	1.45	88.33	17.53	13.09	18.12	8.58	-4478.41	-6.36	-10.93	18.24
V_3	2.87	88.00	17.53	13.05	17.97	8.47	-4472.24	-1.36	-8.86	4.20
V_4	2.67	87.13	16.66	13.20	17.38	8.60	-4117.29	-0.32	-8.98	5.95
V_5	2.92	80.67	15.08	11.87	15.45	7.74	-3641.44	-0.32	-8.99	4.79
V_6	1.87	73.06	14.37	11.12	14.68	7.17	-3677.23	-0.43	-8.80	4.82
V_7	3.64	98.82	18.52	14.39	18.30	8.25	-4501.31	-0.81	-8.63	2.96
V_8	3.33	90.91	16.49	13.02	17.16	9.06	-3924.42	-0.58	-8.55	4.71
V_9	2.42	93.60	18.23	14.53	19.31	9.46	-4593.02	-0.32	-8.74	3.92
V_{10}	2.67	87.13	16.66	13.20	17.38	8.60	-4117.32	-0.30	-8.93	5.03
V_{11}	2.71	94.38	17.53	14.24	18.36	8.76	-4173.12	-0.20	-8.39	6.29
V_{12}	3.44	85.48	15.95	12.99	16.72	8.11	-4001.49	-0.42	-9.01	5.39
V_{13}	2.67	87.13	16.66	13.20	17.38	8.60	-4117.25	-0.27	-8.90	6.00
V_{14}	2.67	87.13	16.66	13.20	17.38	8.60	-4117.25	-0.27	-8.90	6.00
V_{15}	3.44	85.48	15.95	12.99	16.72	8.11	-4001.54	-0.50	-9.03	3.57
V_{16}	2.64	82.37	15.95	12.24	16.39	7.87	-3962.08	-0.30	-8.72	4.05
V_{17}	2.42	93.60	18.23	14.53	19.31	9.46	-4593.13	-0.24	-8.77	6.16
V_{18}	0.62	59.74	11.97	9.48	12.91	6.43	-2974.20	-0.37	-8.99	4.80
V_{19}	2.79	86.87	16.66	12.74	16.91	8.25	-3961.77	-0.69	-9.07	4.23
V_{20}	2.60	87.26	16.66	12.78	17.09	8.39	-4089.49	-0.82	-9.04	7.20
V_{21}	3.06	80.89	15.95	12.17	16.36	7.84	-4112.85	-0.49	-9.04	3.32
V_{22}	1.65	93.38	18.23	14.38	18.91	9.16	-4494.29	-0.49	-8.62	1.02
V_{23}	1.97	95.97	18.23	15.38	19.39	9.52	-4678.04	-0.56	-8.87	2.38
V_{24}	0.93	94.81	18.94	14.79	19.86	9.88	-4814.26	-0.49	-8.47	0.78
V_{25}	1.18	88.34	17.36	13.46	17.93	9.02	-4338.42	-0.50	-8.79	1.17
V_{26}	1.70	93.15	18.23	14.58	19.20	9.38	-4698.53	-0.55	-8.82	2.32
V_{27}	1.13	95.67	19.81	14.65	20.45	9.73	-5169.33	-0.91	-9.31	7.61
V_{28}	1.70	93.15	18.23	14.58	19.20	9.38	-4698.49	-0.51	-8.84	0.78
V_{29}	1.65	100.47	20.68	15.76	21.73	10.11	-5529.13	-1.00	-9.29	6.06
V_{30}	1.65	100.47	20.68	15.76	21.73	10.11	-5529.33	-1.04	-9.08	5.37
V_{31}	1.70	93.15	18.23	14.58	19.20	9.38	-4698.52	-0.53	-8.97	2.44
V_{32}	1.70	93.15	18.23	14.58	19.20	9.38	-4698.52	-0.53	-8.97	2.44
V_{33}	1.65	93.38	18.23	14.38	18.91	9.16	-4494.28	-0.49	-8.74	1.40
V_{34}	1.13	95.67	19.81	14.65	20.45	9.73	-5169.27	-0.85	-9.05	5.12

Table 5. Correlation matrix for antibacterial activity of the synthesized compounds

	$pMIC_{ab}$	log P	MR	$^0\chi^v$	$\kappa\alpha_1$	$\kappa\alpha_2$	Te	LUMO	HOMO	μ
$pMIC_{ab}$	1.000									
log P	-0.018	1.000								
MR	0.686	0.266	1.000							
$^0\chi^v$	0.743	0.135	0.977	1.000						
$\kappa\alpha_1$	0.742	0.014	0.953	0.974	1.000					
$\kappa\alpha_2$	0.764	-0.027	0.924	0.962	0.981	1.000				
Te	-0.746	0.025	-0.913	-0.931	-0.980	-0.947	1.000			
LUMO	0.157	0.149	-0.074	-0.016	-0.105	-0.043	0.160	1.000		
HOMO	0.201	0.155	0.110	0.152	0.054	0.087	0.017	0.899	1.000	
μ	-0.314	0.003	-0.108	-0.184	-0.095	-0.143	0.067	-0.798	-0.843	1.000

During the regression analysis studies it was observed that the response values of compounds V_8, V_{14}, V_{28}, V_{29} and V_{30} were outside the limits of response values of other synthesized 3,4-dimethoxy benzoic acid derivatives. Thus these compounds were designated as outliers and were not included in the data set for QSAR model generation. In multivariate statistics, it is common to define three types of outliers ((Furusjo *et al*., 2006).

- X/Y relation outliers are substances for which the relationship between the descriptors (X variables) and the dependent variables (Y variables) is not the same as in the (rest of the) training data.
- X outliers are substances whose molecular descriptors do not lie in the same range as the (rest of the) training data.
- Y outliers are only defined for training or test samples. They are substances for which the reference value of response is invalid.

As there was no difference in the activity (Table 2) as well as the molecular descriptor range (Table 4) of these outliers when compared to other synthesized 3,4-dimethoxy benzoic acid derivatives, which indicated the fact that these outliers belong to the category of Y outliers (substances for which the reference value of response is invalid). In order to develop mt-QSAR models, initially we calculated the average antibacterial, antifungal and antimicrobial activities values of 3, 4- dimethoxy derivatives which are presented in Table 2. These average antibacterial activity values were correlated with the molecular descriptors of synthesized compounds (Table 5). In general, high colinearity ($r > 0.5$) was observed between different parameters. The high interrelationship was observed between topological parameters, Kier's alpha first and second order shape indices ($\kappa\alpha_1$ and $\kappa\alpha_2$) ($r = 0.981$), and low interrelationship was observed for electronic parameter, dipole moment (μ) and lipophilic parameter (log P) ($r = 0.003$).

Table 6. Correlation of antibacterial, antifungal and antimicrobial activity of the synthesized compounds with calculated molecular descriptors

Descriptors	$pMIC_{ab}$	C_{af}	$pMIC_{am}$
Cos E	-0.237	-0.207	-0.255
log P	-0.018	-0.084	-0.036
MR	0.686	0.605	0.740
$^{0}\chi$	0.719	0.660	0.781
$^{0}\chi^{v}$	0.743	0.611	0.790
$^{1}\chi$	0.719	0.648	0.778
$^{1}\chi^{v}$	0.730	0.628	0.783
$^{2}\chi$	0.698	0.676	0.767
$^{2}\chi^{v}$	0.685	0.621	0.742
$^{3}\chi$	0.626	0.687	0.708
$^{3}\chi^{v}$	0.520	0.513	0.574
κ_1	0.726	0.657	0.786
κ_2	0.740	0.636	0.794
κ_3	0.699	0.660	0.764
$\kappa\alpha_1$	0.742	0.651	0.799
$\kappa\alpha_2$	0.764	0.620	0.810
$\kappa\alpha_3$	0.714	0.646	0.773
R	0.719	0.648	0.778
J	-0.497	-0.507	-0.552
W	0.737	0.660	0.797
Te	-0.746	-0.694	-0.813
Ee	-0.758	-0.651	-0.813
Ne	0.758	0.644	0.811
SA	0.730	0.623	0.782
IP	-0.201	0.326	-0.094
LUMO	0.157	-0.441	0.028
HOMO	0.201	-0.326	0.094
μ	-0.314	0.113	-0.243

Table 7. Observed, predicted and residual antimicrobial activities of the synthesized compounds

Comp.	$pMIC_{ab}$			$pMIC_{af}$			$pMIC_{am}$		
	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.
V_1	0.92	0.91	0.01	1.37	1.31	0.06	1.10	1.08	0.02
V_2	1.12	1.27	-0.15	1.57	1.45	0.12	1.30	1.32	-0.02
V_3	1.12	1.25	-0.13	1.42	1.45	-0.03	1.24	1.32	-0.08
V_4	1.20	1.27	-0.07	1.40	1.42	-0.02	1.28	1.33	-0.05
V_5	1.26	1.17	0.09	1.36	1.38	-0.02	1.30	1.25	0.05
V_6	1.14	1.10	0.04	1.34	1.38	-0.04	1.22	1.21	0.01
V_7	1.35	1.23	0.12	1.45	1.45	0.00	1.39	1.39	0.00
V_8	1.09	1.33	-0.24	1.39	1.40	-0.01	1.21	1.32	-0.11
V_9	1.44	1.37	0.07	1.44	1.46	-0.02	1.44	1.40	0.04
V_{10}	1.30	1.27	0.03	1.40	1.42	-0.02	1.34	1.33	0.01
V_{11}	1.22	1.29	-0.07	1.42	1.42	0.00	1.30	1.38	-0.08
V_{12}	1.11	1.21	-0.10	1.41	1.41	0.00	1.23	1.32	-0.09
V_{13}	1.30	1.27	0.03	1.40	1.42	-0.02	1.34	1.33	0.01
V_{14}	1.68	1.27	0.41	1.48	1.42	0.06	1.60	1.33	0.27
V_{15}	1.41	1.21	0.20	1.41	1.41	0.00	1.41	1.32	0.09
V_{16}	1.08	1.18	-0.10	1.38	1.41	-0.03	1.20	1.28	-0.08
V_{17}	1.34	1.37	-0.03	1.44	1.46	-0.02	1.38	1.40	-0.02
V_{18}	1.05	1.01	0.04	1.25	1.32	-0.07	1.13	1.12	0.01
V_{19}	1.19	1.23	-0.04	1.39	1.41	-0.02	1.27	1.30	-0.03
V_{20}	1.10	1.24	-0.14	1.40	1.42	-0.02	1.22	1.30	-0.08
V_{21}	1.28	1.18	0.10	1.53	1.42	0.11	1.38	1.27	0.11
V_{22}	1.24	1.34	-0.10	1.44	1.45	-0.01	1.32	1.39	-0.07
V_{23}	1.41	1.38	0.03	1.51	1.47	0.04	1.45	1.45	0.00
V_{24}	1.56	1.43	0.13	1.46	1.48	-0.02	1.52	1.41	0.11
V_{25}	1.32	1.32	0.00	1.57	1.44	0.13	1.42	1.34	0.08
V_{26}	1.36	1.36	0.00	1.46	1.47	-0.01	1.40	1.40	0.00
V_{27}	1.38	1.41	-0.03	1.48	1.51	-0.03	1.42	1.41	0.01
V_{28}	1.56	1.36	0.20	1.76	1.47	0.29	1.64	1.40	0.24
V_{29}	1.21	1.45	-0.24	1.51	1.54	-0.03	1.33	1.47	-0.14
V_{30}	1.21	1.45	-0.24	1.51	1.54	-0.03	1.33	1.47	-0.14
V_{31}	1.46	1.36	0.10	1.46	1.47	-0.01	1.46	1.40	0.06
V_{32}	1.36	1.36	0.00	1.46	1.47	-0.01	1.40	1.40	0.00
V_{33}	1.14	1.34	-0.20	1.44	1.45	-0.01	1.26	1.39	-0.13
V_{34}	1.58	1.41	0.17	1.48	1.51	-0.03	1.54	1.41	0.13

Correlation of antibacterial, antifungal and antimicrobial activities of synthesized compounds with their molecular descriptors is given in Table 6. Topological parameter, Kier's alpha second order shape index ($\kappa\alpha_2$) was found to be the dominating descriptor for antibacterial activity of the synthesized compounds (Table 5, Eq. 1).

LR-*mt*-QSAR model for antibacterial activity

$$pMIC_{ab} = 0.121\ \kappa\alpha_2 + 0.230 \quad \text{.....(1)}$$

$n = 29\ r = 0.764\ q^2 = 0.534\ s = 0.102\ F = 37.74$

Here and thereafter, n - number of data points, r - correlation coefficient, q^2-cross validated r^2 obtained by leave one out method, s - standard error of the estimate and F - Fischer statistics. The developed QSAR model for antibacterial activity (Eq. 1) indicated that there is a positive correlation between $\kappa\alpha_2$ and antibacterial activity of the synthesized compounds which means that antibacterial activity values of synthesized compounds will increase with increase in their $\kappa\alpha_2$ values and vice versa. Thus, compound V_1 having lowest $\kappa\alpha_2$ value (5.66, Table 4) is having least antibacterial activity ($pMIC_{ab}$ = 0.92 µM/ml, Table 2). According to Kier, the shape of a molecule may be partitioned into attributes, each describable by the number of bonds of various path lengths. The basis for devising a relative index of shape is given by the relationship of the number of path of length *l* in the molecule *i*, ${}^lP_i$, to some reference values based on molecules with a given number of atoms, *n*, in which the values of ${}^lP$ are maximum and minimum, ${}^lP_{max}$ and ${}^lP_{min}$ (Kier *et al.,* 1999).

The modified kappa shape indices are given by:

$\kappa\alpha_1 = (n + \alpha) (n + \alpha - 1)^2 / ({}^1P_i + \alpha)^2$
$\kappa\alpha_2 = (n + \alpha - 1) (n + \alpha - 2)^2 / ({}^2P_i + \alpha)^2$
$\kappa\alpha_3 = (n + \alpha - 1) (n + \alpha - 3)^2 / ({}^3P_i + \alpha)^2$ n is odd
$\kappa\alpha_3 = (n + \alpha - 3) (n + \alpha - 2)2 / ({}^3P_i + \alpha)^2$ n is even.

The developed QSAR model (Eq. 1) was cross validated by q^2 value (q^2 = 0.534) obtained by leave one out (LOO) method. The value of q^2 more than 0.5 indicated that the model developed is a valid one. According to the recommendations of Golbraikh and Tropsha, 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 (Table 7), the *mt*-QSAR model for antibacterial activity (Eq. 1) is a valid one (Golbraikh and Tropsha*.,* 2002). The plot of predicted $pMIC_{ab}$ against observed $pMIC_{ab}$ (Fig. 2) also favours the developed model expressed by Eq. 1. Further, the plot of observed $pMIC_{ab}$ *vs* residual $pMIC_{ab}$ (Fig. 3) indicated that there was no systemic error in model development as the propagation of error was observed on both sides of zero (Kumar *et al*., 2007). In case of antifungal activity, electronic parameter, total energy (Te, Table 6) was found most dominant in expressing antifungal activity of the synthesized compounds. So, QSAR model for antifungal activity (Eq. 2) was developed using Te.

LR-*mt*-QSAR model for antifungal activity

$$pMIC_{af} = -0.0000854\ Te + 1.068 \quad \text{.....(2)}$$

n = 29 r = 0.694 q^2 = 0.378 s = 0.049 `F = 25.086

Antifungal activity of the synthesized compounds is negatively correlated with their Te values which means that antifungal activity of the synthesized compounds will decrease with increase in their Te values (Tables 3 and 5). The validity and predictability of the QSAR model for antifungal activity *i.e.* Eq. 2 was cross validated by q^2 value (q^2 = 0.378) obtained by leave one out (LOO) method. The value of q^2 less than 0.5 indicated that the developed model is an invalid one. But one should not forget the recommendations of Golbraikh and Tropsha, who 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 (Table 7), the *mt*-QSAR model for antifungal activity Eq. (2) is a valid one (Golbraikh and Tropsha, 2002). Topological parameter, valence zero order molecular connectivity index (${}^0\chi^v$) was found to be most effective in describing antimicrobial activity of the synthesized compounds (Eq. 3, Table 6).

LR-*mt*-QSAR model for antimicrobial activity

$$pMIC_{am} = 0.0546\ {}^0\chi^v + 0.607 \quad \text{.....(3)}$$

n = 29 r = 0.790 q^2 = 0.582 s = 0.068 F=48.82

Antimicrobial activity of the synthesized compounds is positively correlated with valence zero order molecular connectivity index (${}^0\chi^v$) which means that antimicrobial activity of the synthesized compounds will increase with increase in their ${}^0\chi^v$ values (Tables 3 and 5). Compound V_1 having lowest ${}^0\chi^v$ value (8.61, Table 4) is having least antimicrobial activity ($pMIC_{am}$ = 1.10 µM/ml, Table 2). The molecular connectivity index, an adjacency based topological index proposed by Randic is denoted by χ and is defined as sum over all the edges (ij) as per following:

$$\chi = \sum_{i=1}^{n} (V_i V_j)^{-1/2}$$

Where V_i and V_j are the degrees of adjacent vertices i and j and n is the number of vertices in a hydrogen suppressed molecular structure (Lather *et al*., 2005). The topological index χ signifies the degree of branching, connectivity of atoms and unsaturation in the molecule which accounts for variation in activity (Mahiwal *et al*., 2011).

The validity of QSAR model for antimicrobial activity (Eq. 3) is indicated by their high q^2 value (0.582) as well as the low residual values (Table 7). Further, plot of predicted $pMIC_{am}$ against observed $pMIC_{am}$ (Fig.3) also favours the developed model expressed by Eq. 3. The plot of observed $pMIC_{am}$ *vs* residual $pMIC_{am}$ (Fig.3) indicated that there was no systemic error in model development as the propagation of error was observed on both sides of zero. The high residual values observed in case of outliers (V_8, V_{14}, V_{28}, V_{29} and V_{30}) justify their removal while developing QSAR models. It was observed from *mt*-QSAR models (Eq. 1-3) that the antibacterial, antifungal and the overall antimicrobial activities of the synthesized 3,4-dimethoxy benzoic acid derivatives are governed by electronic parameter, total energy (Te) and topological parameters, valence zero order molecular connectivity index (${}^0\chi^v$) and Kier's alpha second order shape index ($\kappa\alpha_3$).

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 one order of magnitude. This is in accordance with results suggested by (Bajaj *et al.,* 2005) who stated that the reliability of the QSAR model lies in its predictive ability even though the activity data are in the narrow range.

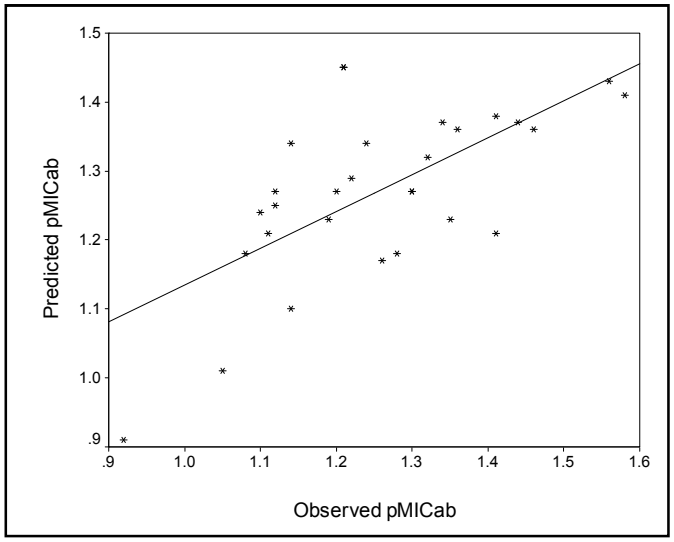


Fig. 1. Plot of observed $pMIC_{ab}$ against predicted $pMIC_{ab}$ by Eq. 1

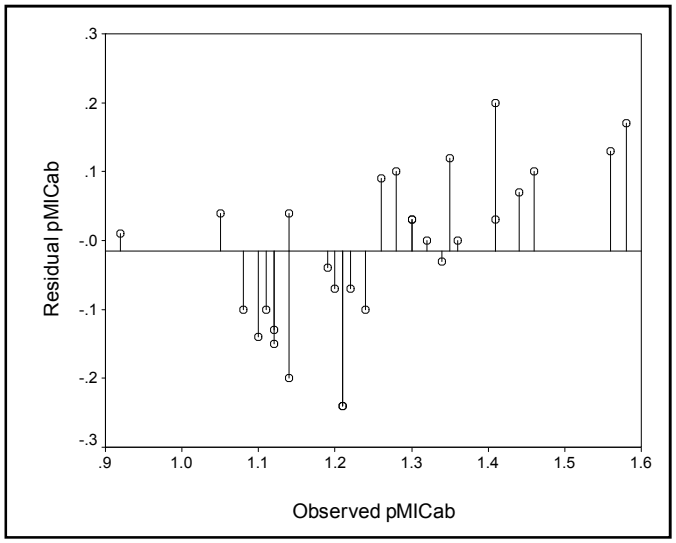


Fig. 2. Plot of observed $pMIC_{ab}$ against residual $pMIC_{ab}$ by Eq. 1

When biological activity data lies in the narrow range, the presence of minimum standard deviation of the biological activity justifies its use in QSAR studies (Narasimhan *et al.* 2007). The minimum standard deviation (Table 2) observed in the antimicrobial activity data justifies its use in QSAR studies.

Conclusion

A number of veratric acid derivatives have been synthesized in moderate to good yield. The title compounds exhibited good *in vitro* antibacterial and antifungal activity. The trend of antimicrobial studies showed that the compounds (V_{15}, V_{31}) explored comparable to superior activity against panel of microorganisms *S. aureus, B. subtilis, E. coli, C. albicans* and *A. niger* (MIC=12.5 µg/ml). The title compounds (V_9, V_{24}) showed encouraged significant antimicrobial activity against *S. aureus* (MIC =6.25µg/ml). Compounds (V_{14}, V_{34}) showed pronounced activity against *B. subtilis* (MIC =6.25 µg/ml). Compounds (V_{14}, V_{28}) were found to have remarkable activity

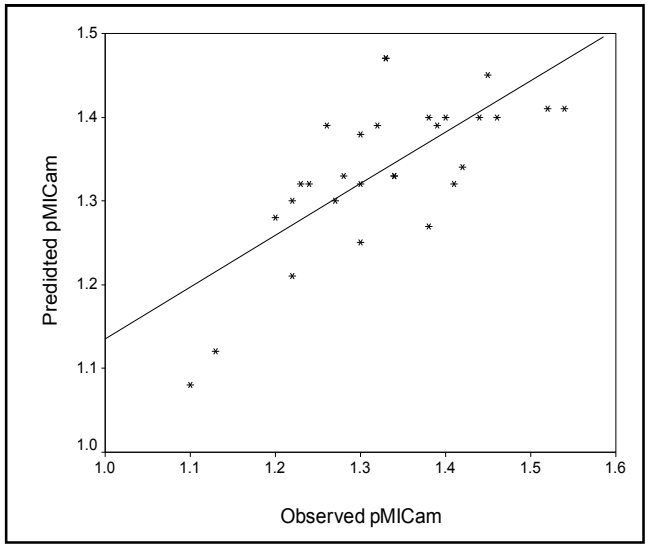


Fig. 3. Plot of observed $pMIC_{am}$ against predicted MIC_{am} by Eq. 3

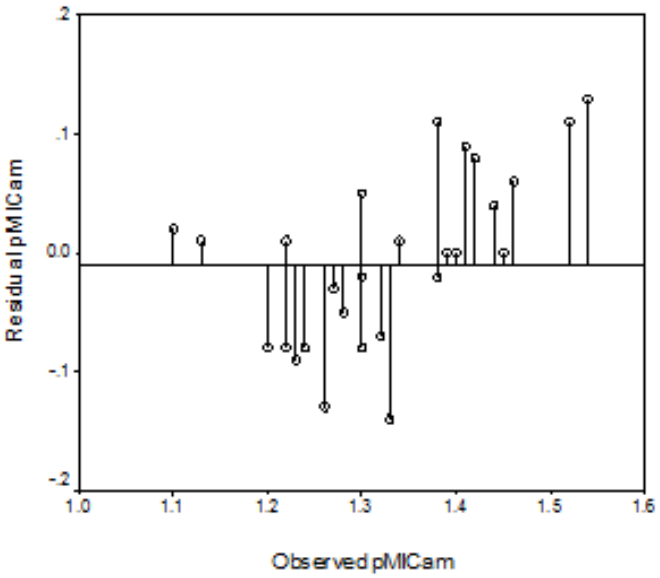


Fig. 4. Plot of observed $pMIC_{am}$ against residual $pMIC_{am}$ by Eq. 3.

against *E.coli* (MIC=6.25µg/ml). Compounds (V_2, V_{21}, V_{25}, V_{28}) demonstrated most significant activity against *C. albicans* (MIC=6.25 µg/ml). Compounds (V_1, V_{28}) detected marked activity against *A. niger* with MIC=6.25 µg/ml. It was also noteworthy that the test compound V_{28} revealed as potent candidate for antifungal activity against *C. albicans* and *A. niger* with MIC= 6.25 µg/ml. Quantitative structure activity relationship studies revealed that antimicrobial activity of these synthesized derivatives against microorganisms under test mainly governed by topological parameter [(valence zero order molecular connectivity index ($^0\chi^v$), kier's alpha second order shape index ($\kappa\alpha_2$)] and electronic parameter [total energy (Te)].

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