

# (Naphthalen-1-yloxy)-acetic acid benzylidene/(1-phenyl-ethylidene)-hydrazide derivatives: synthesis, antimicrobial evaluation, and QSAR studies

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**Abstract** A series of (naphthalen-1-yloxy)-acetic acid hydrazides (**1–36**) was synthesized and screened, in vitro, for antibacterial, antifungal, and antiviral activities. The results of antiviral activity showed that none of the tested compounds was active against viruses at subtoxic concentrations. Further, the antimicrobial screening results demonstrated that compounds having 3,4,5-trimethoxy benzaldehyde (**18**), *o*-Br, *p*-CN (**31**), and *m*-NO<sub>2</sub> acetophenone (**32**) substituents were the most active ones against tested strains. QSAR investigations revealed that multi-target QSAR models were effective in describing the antimicrobial activity.

**Keywords** Hydrazides · Antiviral · Antibacterial · Antifungal · QSAR

## Introduction

The human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS), was identified in the early 1980s (Sakamoto *et al.*, 2007). There are now more than 40 million people living with AIDS worldwide and globally 24.8 million people have died of AIDS since the beginning of epidemic and it is

estimated that 68 million will die of AIDS by 2020 (Gupta *et al.*, 2007). Over the past decade, great strides have been made in the treatment of HIV infection through use of drug combinations known as highly active anti-retroviral therapy (HAART). HAART regimens usually include three or more drugs with inhibitory activity against either HIV reverse transcriptase (RT) or HIV protease. Currently, most HAART regimens include a backbone of two nucleos(t)ide reverse-transcriptase inhibitors (N(t)RTIs) with a third agent added from the non-nucleoside reverse-transcriptase inhibitor (NNRTI) or protease inhibitor classes (PI) (Boojamra *et al.*, 2008). Nevertheless, long-term side effects and virus–drug resistance emergence, together with compliance problems in accurately adhering to the often complicated time schedules of drug uptake, have made it imperative to develop additional drugs, preferably acting at a novel target in the replication cycle of the virus.

Research and development of potent and effective antimicrobial agents represents one of the most important advances in therapeutics, not only in control of serious infections, but also in prevention and treatment of some infectious complications of other therapeutic modalities such as cancer chemotherapy and surgery. Over the past decade, fungal infection become an important complication and a major cause of morbidity and mortality in immunocompromised individuals such as those suffering from tuberculosis, cancer, or AIDS and in organ transplant cases (Rostom *et al.*, 2009). Further, in recent years, much attention has been focused on addressing the problem of multi-drug resistant (MDR) bacteria and fungi resulting from widespread use and misuse of classical antimicrobial agents (Akbas and Berber, 2005). Such serious global health problem demands a renewed effort seeking the development of new antimicrobial agents effective against

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pathogenic microorganisms resistant to currently available treatments.

Quantitative structure–activity relationships (QSARs) are among the most widely used techniques in rational drug design (Bhonsle *et al.*, 2007). The major goal of QSAR is to formulate mathematical relationships between physicochemical properties of compounds and their experimentally determined *in vitro* biological activities. Thus, the derived QSAR model can be subsequently used to predict the biological activities of new derivatives. A good QSAR model both enhances our understanding of the specifics of drug action and provides a theoretical foundation for lead optimization (Kubinyi, 1997). Moreover, QSAR techniques increase the probability of success with reduced time and cost in drug discovery (Pasha *et al.*, 2007).

Hydrazone derivatives have attracted continuing interest over the years because of their wide range of biological activities, viz., antimicrobial (Bayrak *et al.*, 2009), antimycobacterial (Nayyar *et al.*, 2007), antitumour (Lembege *et al.*, 2008), anti-inflammatory (Bhandari *et al.*, 2008), trypanocidal (Leite *et al.*, 2006), antiviral (Osama *et al.*, 2009), and antimalarial activities (Gemma *et al.*, 2006).

Therefore, the target compounds were rationalized so as to comprise the hydrazone pharmacophores that are believed to be responsible for the biological significance of some relevant chemotherapeutic agents. The substitution pattern of such hydrazone derivatives was carefully selected so as to confer a different electronic environment to the molecules. We described previously the antimicrobial profile of hydrazide–hydrazone derivatives of cinnamic acid, benzoic acid, and their derivatives, some of which have exhibited a remarkable antimicrobial activity (Kumar *et al.*, 2008, 2009, 2010a, b). Motivated by the aforementioned findings and with the aim of obtaining new antimicrobial compounds we hereby report the synthesis, antiviral, antibacterial, antifungal, and QSAR studies of (naphthalen-1-yloxy)-acetic acid benzylidene/(1-phenyl-ethylidene)-hydrazide derivatives.

## Experimental

Melting points were determined in open glass capillaries on a sonar melting point apparatus and are uncorrected. Reaction progress was monitored by thin layer chromatography on silica gel sheets (Merck silica gel-G). <sup>1</sup>H nuclear magnetic resonance (<sup>1</sup>H NMR) spectra were recorded on Bruker Avance II 400 NMR spectrometer (400 MHz) at 298 K, in appropriate deuterated solvents. Chemical shifts were reported as  $\delta$  (ppm) relative to tetramethylsilane (TMS) as internal standard. Infrared (IR)

spectra were recorded as KBr pellet on Perkin Elmer FTIR spectrometer. The wave number is given in  $\text{cm}^{-1}$ .

General procedure for synthesis of (naphthalen-1-yloxy)-acetic acid benzylidene/(1-phenyl-ethylidene)-hydrazides (**1–36**)

The mixture of 2-naphthoxy acetic acid (0.08 mol) and ethanol (0.80 mol) was refluxed in the presence of a few drops of concentrated sulfuric acid till the completion of reaction. Then the reaction mixture was added to 200 ml ice cold water and the residual acid was removed by treatment with sodium bicarbonate. The ester formed was extracted with ether. The ether layer was separated which on evaporation yielded the crude ester, which was recrystallized from alcohol. Hydrazine hydrate (99%) (0.015 mol) was added in ethanolic solution of ester (0.01 mol) synthesized above and refluxed for 8 h. The reaction mixture was then cooled and the precipitates of acid hydrazide were filtered off, washed with water, dried, and recrystallized from ethanol. Then the solution of acid hydrazide (0.01 mol) and appropriate aldehydes/acetophenones (0.01 mol) in ethanol was refluxed for 3–4 h. The precipitated title compounds were then filtered off, washed with water, and recrystallized from ethanol.

(*Naphthalen-1-yloxy*)-acetic acid (2-chloro-benzylidene)-hydrazide (**2**)

Mp (°C) 145–148; Yield 85.4%; <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta$  ppm: 11.60 (s, 1H, NH), 8.46 (s, 1H, CH of N=CH), 7.29–8.16 (m, 11H, ArH), 5.24 (s, 2H, CH<sub>2</sub>); IR (KBr pellets):  $\text{cm}^{-1}$  3200.95 (N–H str.), 3053.47 (C–H str., aromatic), 2915.03 (C–H str., aliphatic), 1667.80 (C=O str.), 1252.55 (C=C str., aromatic), 755.20 (C–Cl str.), 835.16 (C–H out-of-plane bending, naphthalene).

(*Naphthalen-1-yloxy*)-acetic acid (3-chloro-benzylidene)-hydrazide (**3**)

Mp (°C) 183–186; Yield 68.8%; <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta$  ppm: 11.75 (s, 1H, NH), 8.01 (s, 1H, CH of N=CH), 7.20–7.81 (m, 11H, ArH), 5.26 (s, 2H, CH<sub>2</sub>); IR (KBr pellets):  $\text{cm}^{-1}$  3201.00 (N–H str.), 3083.34 (C–H str., aromatic), 2968.58 (C–H str., aliphatic), 1682.96 (C=O str.), 1269.22 (C=C str., aromatic), 757.09 (C–Cl str.), 820.75 (C–H out-of-plane bending, naphthalene).

(*Naphthalen-1-yloxy*)-acetic acid (4-chloro-benzylidene)-hydrazide (**4**)

Mp (°C) 203–206; Yield 69.7%; <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta$  ppm: 11.70 (s, 1H, NH), 8.01 (s, 1H, CH of

N=CH), 7.18–7.82 (m, 11H, ArH), 5.24 (s, 2H, CH<sub>2</sub>); IR (KBr pellets): cm<sup>-1</sup> 3172.07 (N–H str.), 3078.52 (C–H str., aromatic), 2959.90 (C–H str., aliphatic), 1684.89 (C=O str.), 1271.14 (C=C str., aromatic), 748.41 (C–Cl str.), 819.78 (C–H out-of-plane bending, naphthalene).

*(Naphthalen-1-yloxy)-acetic acid (4-bromo-benzylidene)-hydrazide (7)*

Mp (°C) 161–164; Yield 85.6%; <sup>1</sup>H NMR (400 MHz, DMSO) δ ppm: 11.73 (s, 1H, NH), 8.02–8.33 (s, 1H, CH of N=CH), 7.22–7.90 (m, 11H, ArH), 4.80–5.28 (s, 2H, CH<sub>2</sub>); IR (KBr pellets): cm<sup>-1</sup> 3176.00 (N–H str.), 3055.50 (C–H str., aromatic), 2961.65 (C–H str., aliphatic), 1686.97 (C=O str.), 1426.74 (C=C str., aromatic), 809.34 (C–H out-of-plane bending, naphthalene), 510.10 (C–Br str.).

*(Naphthalen-1-yloxy)-acetic acid (4-fluoro-benzylidene)-hydrazide (8)*

Mp (°C) 182–185; Yield 68.9%; <sup>1</sup>H NMR (400 MHz, DMSO) δ ppm: 11.66 (s, 1H, NH), 8.03 (s, 1H, CH of N=CH), 7.14–7.83 (m, 11H, ArH), 5.24 (s, 2H, CH<sub>2</sub>); IR (KBr pellets): cm<sup>-1</sup> 3179.79 (N–H str.), 3079.49 (C–H str., aromatic), 2969.82 (C–H str., aliphatic), 1683.93 (C=O str.), 1498.75 (C=C str., aromatic), 1262.46 (C–F str.), 755.16 (C–H out-of-plane bending, naphthalene).

*(Naphthalen-1-yloxy)-acetic acid (2-nitro-benzylidene)-hydrazide (9)*

Mp (°C) 167–170; Yield 81.7%; <sup>1</sup>H NMR (400 MHz, DMSO) δ ppm: 11.97 (s, 1H, NH), 8.48 (s, 1H, CH of N=CH), 7.32–8.12 (m, 11H, ArH), 5.23 (s, 2H, CH<sub>2</sub>); IR (KBr pellets): cm<sup>-1</sup> 3201.97 (N–H str.), 3086.24 (C–H str., aromatic), 2941.57 (C–H str., aliphatic), 1697.43 (C=O str.), 1260.54 (C=C str., aromatic), 1519.97 (NO<sub>2</sub> asym. str.), 1425.46 (NO<sub>2</sub> sym. str.), 749.38 (C–H out-of-plane bending, naphthalene).

*(Naphthalen-1-yloxy)-acetic acid (3-nitro-benzylidene)-hydrazide (10)*

Mp (°C) 168–171; Yield 73.6%; <sup>1</sup>H NMR (400 MHz, DMSO) δ ppm: 11.89 (s, 1H, NH), 8.52 (s, 1H, CH of N=CH), 7.22–8.49 (m, 12H, ArH), 5.29 (s, 2H, CH<sub>2</sub>); IR (KBr pellets): cm<sup>-1</sup> 3186.54 (N–H str.), 3039.94 (C–H str., aromatic), 2923.25 (C–H str., aliphatic), 1679.11 (C=O str.), 1266.32 (C=C str., aromatic), 1536.37 (NO<sub>2</sub> asym. str.), 1352.16 (NO<sub>2</sub> sym. str.), 821.71 (C–H out-of-plane bending, naphthalene).

*(Naphthalen-1-yloxy)-acetic acid (4-nitro-benzylidene)-hydrazide (11)*

Mp (°C) 193–196; Yield 71.4%; <sup>1</sup>H NMR (400 MHz, DMSO) δ ppm: 11.86 (s, 1H, NH), 8.09 (s, 1H, CH of N=CH), 7.17–7.95 (m, 11H, ArH), 5.27 (s, 2H, CH<sub>2</sub>); IR (KBr pellets): cm<sup>-1</sup> 3220.22 (N–H str.), 3061.68 (C–H str., aromatic), 2950.50 (C–H str., aliphatic), 1676.54 (C=O str.), 1563.80 (C=C str., aromatic), 1531.63 (NO<sub>2</sub> asym. str.), 1357.2 (NO<sub>2</sub> sym. str.), 800.32 (C–H out-of-plane bending, naphthalene), 510.10 (C–Br str.).

*(Naphthalen-1-yloxy)-acetic acid (2-hydroxy-benzylidene)-hydrazide (12)*

Mp (°C) 201–203; Yield 77.8%; <sup>1</sup>H NMR (400 MHz, DMSO) δ ppm: 11.85 (s, 1H, NH), 11.17 (s, 1H, OH), 8.57 (s, 1H, CH of N=CH), 6.86–7.84 (m, 11H, ArH), 4.81 (s, 2H, CH<sub>2</sub>); IR (KBr pellets): cm<sup>-1</sup> 3380.60 (O–H str.), 3178.70 (N–H str.), 3084.30 (C–H str., aromatic), 2921.15 (C–H str., aliphatic), 1680.53 (C=O str.), 1471.40 (C=C str., aromatic), 1255.70 (C–O str., phenol), 755.80 (C–H out-of-plane bending, naphthalene).

*(Naphthalen-1-yloxy)-acetic acid (4-hydroxy-benzylidene)-hydrazide (14)*

Mp (°C) 212–215; Yield 69.3%; <sup>1</sup>H NMR (400 MHz, DMSO) δ ppm: 11.35 (s, 1H, NH), 9.57 (s, 1H, OH), 7.92 (s, 1H, CH of N=CH), 7.15–7.78 (m, 11H, ArH), 5.21 (s, 2H, CH<sub>2</sub>); IR (KBr pellets): cm<sup>-1</sup> 3296.49 (N–H str.), 3043.80 (C–H str., aromatic), 2932.89 (C–H str., aliphatic), 1699.36 (C=O str.), 1598.09 (C=C str., aromatic), 1245.10 (C–O str., phenol), 836.18 (C–H out-of-plane bending, naphthalene).

*(Naphthalen-1-yloxy)-acetic acid (3,4-dimethoxy-benzylidene)-hydrazide (17)*

Mp (°C) 165–168; Yield 70.2%; <sup>1</sup>H NMR (400 MHz, DMSO) δ ppm: 11.52 (s, 1H, NH), 7.95 (s, 1H, CH of N=CH), 6.89–7.91 (m, 10H, ArH), 5.24 (s, 2H, CH<sub>2</sub>), 3.85–3.86 (s, 6H, OCH<sub>3</sub>); IR (KBr pellets): cm<sup>-1</sup> 3191.36 (N–H str.), 3060.20 (C–H str., aromatic), 2942.53 (C–H str., aliphatic), 1680.07 (C=O str.), 1599.06 (C=C str., aromatic), 1244.14 (C–O–C asym. str.), 1165.05 (C–O–C sym. str.), 735.87 (C–H out-of-plane bending, naphthalene).

*(Naphthalen-1-yloxy)-acetic acid (4-hydroxy-3-methoxy-benzylidene)-hydrazide (19)*

Mp (°C) 138–141; Yield 78.6%; <sup>1</sup>H NMR (400 MHz, DMSO) δ ppm: 11.51 (s, 1H, NH), 9.58 (s, 1H, OH),

7.93–8.24 (s, 1H, CH of N=CH), 6.28–7.90 (m, 10H, ArH), 4.78–5.27 (s, 2H, CH<sub>2</sub>), 3.80–3.81 (s, 3H, OCH<sub>3</sub>); IR (KBr pellets): cm<sup>-1</sup> 3464.09 (OH str.), 3190.36 (N–H str.), 3090.73 (C–H str., aromatic), 2972.80 (C–H str., aliphatic), 1682.65 (C=O str.), 1521.46 (C=C str., aromatic), 1248.30 (C–O–C asym. str.), 1158.84 (C–O–C sym. str.), 761.28 (C–H out-of-plane bending, naphthalene).

*(Naphthalen-1-yloxy)-acetic acid (4-methyl-benzylidene)-hydrazide (23)*

Mp (°C) 196–199; Yield 87.5%; <sup>1</sup>H NMR (400 MHz, DMSO) δ ppm: 11.54 (s, 1H, NH), 7.99 (s, 1H, CH of N=CH), 7.16–7.87 (m, 11H, ArH), 5.23 (s, 2H, CH<sub>2</sub>), 2.14 (s, 3H, CH<sub>3</sub>); IR (KBr pellets): cm<sup>-1</sup> 3180.00 (N–H str.), 3053.40 (C–H str., aromatic), 2963.15 (C–H str., aliphatic), 1689.40 (C=O str.), 1401.59 (C=C str., aromatic), 777.38 (C–H out-of-plane bending, naphthalene).

*(Naphthalen-1-yloxy)-acetic acid [1-(4-chloro-phenyl)-ethylidene]-hydrazide (28)*

Mp (°C) 152–155; Yield 78.5%; <sup>1</sup>H NMR (400 MHz, DMSO) δ ppm: 10.91 (s, 1H, NH), 7.15–7.80 (m, 12H, ArH), 5.26 (s, 2H, CH<sub>2</sub>), 2.30 (s, 3H, CH<sub>3</sub>); IR (KBr pellets): cm<sup>-1</sup> 3363.61 (N–H str.), 3050.90 (C–H str., aromatic), 2924.11 (C–H str., aliphatic), 1694.21 (C=O str.), 1434.48 (C=C str., aromatic), 827.88 (C–H out-of-plane bending, naphthalene), 744.88 (C–Cl str.).

*(Naphthalen-1-yloxy)-acetic acid [1-(4-bromo-phenyl)-ethylidene]-hydrazide (30)*

Mp (°C) 175–178; Yield 79.6%; <sup>1</sup>H NMR (400 MHz, DMSO) δ ppm: 10.72 (s, 1H, NH), 7.52–7.84 (m, 12H, ArH), 5.26 (s, 2H, CH<sub>2</sub>), 2.31 (s, 3H, CH<sub>3</sub>); IR (KBr pellets): cm<sup>-1</sup> 3190.34 (N–H str.), 3061.14 (C–H str., aromatic), 2964.45 (C–H str., aliphatic), 1686.35 (C=O str.), 1406.80 (C=C str., aromatic), 804.10 (C–H out-of-plane bending, naphthalene), 508.70 (C–Br str.).

*(Naphthalen-1-yloxy)-acetic acid [1-(3-nitro-phenyl)-ethylidene]-hydrazide (32)*

Mp (°C) 170–173; Yield 65.7%; <sup>1</sup>H NMR (400 MHz, DMSO) δ ppm: 11.12 (s, 1H, NH), 8.62 (s, 1H, CH of N=CH), 7.16–7.87 (m, 11H, ArH), 5.31 (s, 2H, CH<sub>2</sub>), 2.13 (s, 3H, CH<sub>3</sub>); IR (KBr pellets): cm<sup>-1</sup> 3380.40 (N–H str.), 3061.16 (C–H str., aromatic), 2918.42 (C–H str., aliphatic), 1713.83 (C=O str.), 1517.08 (NO<sub>2</sub> asym. str.), 1353.12 (NO<sub>2</sub> sym. str.), 1248.00 (C=C str., aromatic), 759.02 (C–H out-of-plane bending, naphthalene).

*(Naphthalen-1-yloxy)-acetic acid [1-(2-hydroxy-phenyl)-ethylidene]-hydrazide (34)*

Mp (°C) 154–157; Yield 77.5%; <sup>1</sup>H NMR (400 MHz, DMSO) δ ppm: 12.97 (s, 1H, OH), 11.07 (s, 1H, NH), 6.81–7.77 (m, 12H, ArH), 4.86 (s, 2H, CH<sub>2</sub>), 2.37 (s, 3H, CH<sub>3</sub>); IR (KBr pellets): cm<sup>-1</sup> 3472.02 (O–H str.), 3379.43 (N–H str.), 3050.55 (C–H str., aromatic), 2927.10 (C–H str., aliphatic), 1692.61 (C=O str.), 1384.95 (C=C str., aromatic), 763.84 (C–H out-of-plane bending, naphthalene).

*(Naphthalen-1-yloxy)-acetic acid [1-(4-hydroxy-phenyl)-ethylidene]-hydrazide (35)*

Mp (°C) 182–185; Yield 80.5%; <sup>1</sup>H NMR (400 MHz, DMSO) δ ppm: 10.65 (s, 1H, NH), 9.47 (s, 1H, OH), 6.77–7.94 (m, 12H, ArH), 5.25 (s, 2H, CH<sub>2</sub>), 2.12 (s, 3H, CH<sub>3</sub>); IR (KBr pellets): cm<sup>-1</sup> 3382.26 (N–H str.), 3180.00 (N–H str.), 3051.96 (C–H str., aromatic), 1695.59 (C=O str.), 1457.46 (C=C str., aromatic), 1255.93 (C–O str., phenol), 753.85 (C–H out-of-plane bending, naphthalene).

Evaluation of antimicrobial activity (determination of minimum inhibitory concentration)

*Antibacterial assay*

A 24-h fresh culture was obtained by inoculation of respective bacteria in double strength nutrient broth I.P. (Pharmacopoeia of India, 2007) followed by incubation at 37 ± 1°C. The stock solution (100 µg/ml) of synthesized substituted benzylidene hydrazide derivatives was serially diluted in tubes (Cappucino and Sherman, 1999) containing 1 ml of sterile double strength nutrient broth I.P. to get a concentration of 50 to 3.125 µg/ml and then inoculated with 100 µl of suspension of respective organisms in sterile saline (*S. aureus*, *B. subtilis* and *E. coli*). The inoculated tubes were incubated at 37 ± 1°C for 24 h and minimum inhibitory concentrations (MICs) were determined.

*Antifungal assay*

The antifungal activity of synthesized hydrazide derivatives against the fungal species *C. albicans* and *A. niger* was determined by serial dilution method similar to antibacterial assay using Sabouraud dextrose broth I.P. The inoculated tubes were incubated at 37 ± 1 and 25 ± 1°C for a period of 2 and 7 days in case of *C. albicans* and *A. niger*, respectively.

## QSAR studies

### Theory of step wise multiple linear regression (MLR)

For the construction of QSAR models, most often a linear model is sought, which can be expressed as the following equation (Damme and Bultinck, 2010)

$$A = k_1 * D_1 + k_2 * D_2 + \text{constant}$$

Here  $A$  stands for the biological activity,  $D_1$  and  $D_2$  stands for the value of different molecular descriptor retained in the QSAR,  $k_1$  and  $k_2$  is the regression constant belonging to descriptor  $D_1$  and  $D_2$ , and  $C$  is a constant. The core machinery of deriving a QSAR are statistical algorithms that pick the most discriminating descriptors for each problem from a large pool of possible descriptors and yield the coefficients  $k$ . Obviously, in order to develop a QSAR, one first needs a set of molecules for which the activities are known as well as a set of descriptors from which usually only a small fraction will be kept in the final QSAR. Obviously, the better one can describe the molecule in terms of a set of molecular descriptors, the bigger the chance of a better QSAR. As a consequence, there has been a lot of research on the development of new descriptors that may be better at representing different aspects of molecular behavior, e.g., reactivity. Note that extending the pool of descriptors from which to pick the significant descriptors for the problem considered is fruitful, provided the descriptors are not correlated. Hence, increasing the number of descriptors retained in the QSAR model has to be done with great care to avoid overfitting (Damme and Bultinck, 2010).

### Descriptor generation

A Pentium IV personal computer (CPU at 1.96 GHz) with windows XP operating system was used. The two-dimensional structures of synthesized hydrazide derivatives were drawn by chemoffice 6.0 software to calculate whole molecular structure-based descriptors. The structure of molecules are first pre-optimized with the Molecular Mechanics Force Field (MM<sup>+</sup>) procedure included in Hyperchem 6.03 (Hyperchem 6.0, 1993) and the resulting geometries are further refined by means of the semiempirical method PM3 (parametric Method-3). The structures were minimized with the conjugate gradient method until the gradient is <0.01 kcal/mol Å. This lowest energy structure was used for each molecule to calculate physicochemical properties using TSAR 3.3 software for Windows (TSAR 3D Version 3.3, 2000).

### Data screening and model building

The selected descriptors from each class and the experimental data were analyzed by the stepwise regression SPSS (version

12.0) software package (SPSS for Windows, 1999). The calculated descriptors were collected in a data matrix whose number of rows and columns were the number of molecules and descriptors, respectively. Linear regression (LR) and MLRs were used to derive the QSAR equations and feature selection was performed using the SPSS software package. The resulted models were validated by leave-one-out (LOO) cross-validation method (Schaper, 1999). The statistical qualities of the equations were judged by the parameters like correlation coefficient ( $r$ ), cross-validated  $r^2$  ( $q^2$ ), standard error of estimate ( $s$ ), and variance ratio ( $F$ ) at specified degrees of freedom and some other statistical parameters were employed to confirm their predictability and robustness.

### Calculation of statistical parameters

The developed QSAR models were validated by the calculation of following statistical parameters: probable error of the coefficient of correlation (PE), least square error (LSE), Friedman's lack of fit measure (LOF), standard error of prediction (SEP), quality value ( $Q$ ), and sum of squares of response values (SSY) (Mandloi *et al.*, 2005; Pinheiro *et al.*, 2004).

These parameters were calculated from the following equations.

$$PE = 2(1 - r^2)/3\sqrt{n}$$

where  $r$  correlation coefficient and  $n$  number of compounds used.

$$LSE = \sum (Y_{\text{obs}} - Y_{\text{calc}})^2$$

where  $Y_{\text{obs}}$  and  $Y_{\text{calc}}$  are the observed and calculated values.

$$LOF = LSE / \{1 - (C + d \cdot p/n)\}^2$$

where LSE, least square error;  $C$  number of descriptors +1;  $p$ , number of independent parameters;  $n$ , number of compounds used;  $d$ , smoothing parameter which controls the bias in the scoring factor between equations with different number of terms and was kept 1.0.

$$SEP = \sqrt{LSE/n}$$

The quality value,  $Q$  is given by

$$Q = r/Se$$

where  $Q$ , quality value;  $r$ , correlation coefficient; and  $Se$ , standard error.

The predictive ability of QSAR models was also quantified in terms of  $q^2$ , which is defined as

$$q^2 = 1 - \left\{ \frac{\sum (Y_{\text{obs}} - Y_{\text{calc}})^2}{\sum (Y_{\text{obs}} - Y_{\text{mean}})^2} \right\}$$

The low value of PE, LSE, LOF, and SEP and high value of  $Q$  and  $q^2$  are the essential criteria for qualifying the model as the best one.

Variation inflation factor (Goodarzi *et al.*, 2010) is employed to determine the multicollinearity between the physicochemical parameters. The VIF and tolerance value is calculated as

$$\text{VIF} = 1/1 - r^2$$

$$\text{Tolerance} = 1/\text{VIF}$$

where  $r^2$  is the squared multiple correlation coefficient of one parameter effect on the remaining parameter.

In the resubstitution test, three statistical parameters ( $r$ , RMSE, and  $e^-$ ) were used to evaluate the performance (Zhao *et al.*, 2007). The regression coefficient  $r$  is defined by

$$\text{Regression coefficient } (r) = \sqrt{\left\{1 - \left(\frac{\sum (Y_{\text{calc}} - Y_{\text{obs}})^2}{\sum (Y_{\text{calc}} - Y_{\text{mean}})^2}\right)\right\}}$$

The root mean square of errors (RMSE) is calculated with equation

$$\text{RMSE} = \sqrt{\left\{\sum (Y_{\text{calc}} - Y_{\text{obs}})^2/n\right\}}$$

To illustrate the predictive accuracy more explicitly, the  $e^-$  (absolute average error) is defined by equation

$$e^- = \sum |Y_{\text{calc}} - Y_{\text{obs}}|/n$$

#### Evaluation of anti-HIV activity

The anti-HIV activity and cytotoxicity were evaluated against wild-type HIV-1 (IIIB) and HIV-2 (ROD) in MT-4 cell cultures using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (Pauwels *et al.*, 1988). Briefly, virus stocks were titrated in MT-4 cells and expressed as the 50% cell culture infective dose (CCID<sub>50</sub>). MT-4 cells were suspended in culture medium at  $1 \times 10^5$  cells/ml and infected with HIV at a multiplicity of infection of 0.02. Immediately after viral infection, 100  $\mu$ l of the cell suspension was placed in each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. After 4-day incubation at 37°C, the number of viable cells was determined using the MTT method. Compounds were tested in parallel for their cytotoxic effects in uninfected MT-4 cell cultures.

#### Antiviral assays

The antiviral assays [except anti-human immunodeficiency virus (HIV) assays] were based on inhibition of virus-induced cytopathicity in HEL [varicella-zoster virus (TK<sup>+</sup> VZV strain and TK<sup>-</sup> VZV strain) and cytomegalovirus (AD-169 strain and Davis strain)] cell culture. Confluent cell cultures in microtiter 96-well plates were inoculated

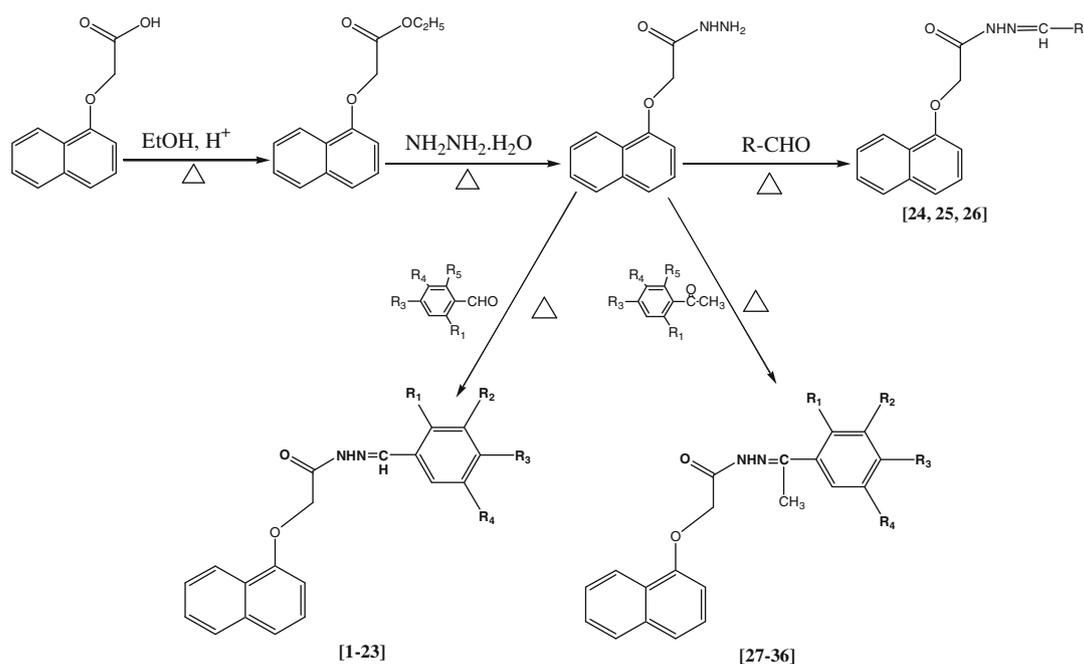
with 100 cell culture inhibitory dose-50 (CCID<sub>50</sub>) of virus (1 CCID<sub>50</sub> being the virus dose to infect 50% of the cell cultures) in the presence of varying concentrations (100, 20, 4, ...  $\mu$ g/ml) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds.

## Results and discussion

### Chemistry

The synthesis of the intermediate and target compounds (**1–36**) was performed according to reactions outlined in Scheme 1. The 2-naphthoxy acetic acid was refluxed with ethanol in the presence of a few drops of sulfuric acid to yield the ethyl ester. The ethyl ester was refluxed with hydrazine hydrate in ethanol to yield the acid hydrazide, which was then condensed with corresponding aromatic aldehydes (**1–26**)/acetophenones (**27–36**) to yield the target substituted hydrazides. The physicochemical characteristics of synthesized compounds are presented in Table 1. <sup>1</sup>H NMR and IR spectral data are in agreement with the proposed structures of all newly synthesized compounds.

The appearance of singlet signal ranging from  $\delta$  10.65–11.97 ppm in compounds **1–36** confirmed the presence of NH group of synthesized hydrazide derivatives. The presence of aromatic hydrogens was confirmed by the multiplet signal in the range of  $\delta$  7.0–9.0 ppm. The one proton of N=CH group appeared as singlet in the range of  $\delta$  7.95–8.48 ppm in the synthesized compounds confirmed the formation of hydrazone derivatives, which is formed by removal of two hydrogen molecules from the hydrazide derivatives and one oxygen molecule from benzaldehydes or acetophenones. The appearance of singlet signals around  $\delta$  5.25 ppm revealed the presence of CH<sub>2</sub> group in synthesized derivatives. The presence of singlet signal around  $\delta$  2.12–2.37 ppm confirmed the presence of CH<sub>3</sub> group of acetophenones in the synthesized compounds (**27–36**). Further, in compounds **17** and **19**, singlet signal at  $\delta$  3.85–3.86 ppm and  $\delta$  3.80–3.81 ppm depicted the presence of 6 and 3 hydrogens of methoxy groups, respectively. The singlet signal at  $\delta$  11.17, 9.57, 9.58, 12.97, and 9.47 ppm in compounds **12**, **14**, **19**, **34**, and **35** confirmed the presence of hydroxyl group in their structures. The three protons appeared as singlet at  $\delta$  2.14 ppm confirmed the presence of methyl group in compound **23**. The highest  $\delta$  11.97 ppm and least  $\delta$  10.65 ppm of NH proton indicated the presence of electron withdrawing NO<sub>2</sub> (causes deshielding of protons) group and electron donating OH (causes shielding of protons) group in compounds **9** and **35**, respectively.



Comp.	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R	Comp.	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R
1	H	H	H	H	-	19	H	OH	OCH <sub>3</sub>	H	-
2	Cl	H	H	H	-	20	H	OH	OC <sub>2</sub> H <sub>5</sub>	H	-
3	H	Cl	H	H	-	21	H	OCH <sub>3</sub>	OH	OCH <sub>3</sub>	-
4	H	H	Cl	H	-	22	H	H	SCH <sub>3</sub>	H	-
5	Br	H	H	H	-	23	H	H	CH <sub>3</sub>	H	-
6	H	Br	H	H	-	24	-	-	-	-	
7	H	H	Br	H	-	25	-	-	-	-	
8	H	H	F	H	-	26	-	-	-	-	
9	NO <sub>2</sub>	H	H	H	-	27	H	H	H	H	-
10	H	NO <sub>2</sub>	H	H	-	28	H	H	Cl	H	-
11	H	H	NO <sub>2</sub>	H	-	29	Br	H	H	H	-
12	OH	H	H	H	-	30	H	H	Br	H	-
13	H	OH	H	H	-	31	Br	H	CN	H	-
14	H	H	OH	H	-	32	H	NO <sub>2</sub>	H	H	-
15	H	H	OCH <sub>3</sub>	H	-	33	H	H	NO <sub>2</sub>	H	-
16	OCH <sub>3</sub>	H	H	OCH <sub>3</sub>	-	34	OH	H	H	H	-
17	H	OCH <sub>3</sub>	OCH <sub>3</sub>	H	-	35	H	H	OH	H	-
18	H	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	-	36	OH	H	OH	H	-

**Scheme 1** Scheme for the synthesis of substituted (naphthalen-1-yloxy)-acetic acid benzylidene/(1-phenyl-ethylidene)-hydrazides

Furthermore, the absence of singlet signal corresponding to COOH in the NMR spectra of synthesized compounds (1–36) supports the complete reaction of 2-naphthoxyacetic acid.

The presence of C=O functional group was marked by the appearance of a stretching band around 1700 cm<sup>-1</sup>, which is the characteristic of an amide linkage. The presence of IR band around 800 cm<sup>-1</sup> showed the presence of naphthalene

moiety in the synthesized compounds. The appearance of stretching band around 3200–3400 cm<sup>-1</sup> indicated the presence of NH linkage of amide bond hydrazide derivatives. The presence of peaks slightly above and below 3000 cm<sup>-1</sup> indicated the presence of aromatic and aliphatic protons in the synthesized compounds, respectively. The characteristic C–Cl str. bands were observed at 755.20, 757.09, 748.41, and 744.88 cm<sup>-1</sup> in compounds 2, 3, 4, and 28, respectively.

**Table 1** Physicochemical properties of synthesized (naphthalen-1-yloxy)-acetic acid benzylidene/(1-phenyl-ethylidene)-hydrazide derivatives

Comp.	Mol. formula	Mol. wt.	Mp (°C)	R <sub>f</sub> <sup>a</sup>	% Yield
1	C <sub>19</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub>	304	176–179	0.42	86.9
2	C <sub>19</sub> H <sub>15</sub> ClN <sub>2</sub> O <sub>2</sub>	339	145–148	0.50	85.4
3	C <sub>19</sub> H <sub>15</sub> ClN <sub>2</sub> O <sub>2</sub>	339	183–186	0.61	68.8
4	C <sub>19</sub> H <sub>15</sub> ClN <sub>2</sub> O <sub>2</sub>	339	203–206	0.54	69.7
5	C <sub>19</sub> H <sub>15</sub> BrN <sub>2</sub> O <sub>2</sub>	383	138–141	0.62	74.5
6	C <sub>19</sub> H <sub>15</sub> BrN <sub>2</sub> O <sub>2</sub>	383	140–143	0.74	72.4
7	C <sub>19</sub> H <sub>15</sub> BrN <sub>2</sub> O <sub>2</sub>	383	161–164	0.37	85.6
8	C <sub>19</sub> H <sub>15</sub> FN <sub>2</sub> O <sub>2</sub>	322	182–185	0.48	68.9
9	C <sub>19</sub> H <sub>15</sub> N <sub>3</sub> O <sub>4</sub>	349	167–170	0.69	81.7
10	C <sub>19</sub> H <sub>15</sub> N <sub>3</sub> O <sub>4</sub>	349	168–171	0.68	73.6
11	C <sub>19</sub> H <sub>15</sub> N <sub>3</sub> O <sub>4</sub>	349	193–196	0.45	71.4
12	C <sub>19</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub>	320	201–204	0.77	77.8
13	C <sub>19</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub>	320	170–173	0.69	82.1
14	C <sub>19</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub>	320	212–215	0.47	69.3
15	C <sub>20</sub> H <sub>18</sub> N <sub>2</sub> O <sub>3</sub>	334	163–166	0.60	80.5
16	C <sub>21</sub> H <sub>20</sub> N <sub>2</sub> O <sub>4</sub>	364	163–166	0.79	62.6
17	C <sub>21</sub> H <sub>20</sub> N <sub>2</sub> O <sub>4</sub>	364	165–168	0.58	70.2
18	C <sub>22</sub> H <sub>22</sub> N <sub>2</sub> O <sub>5</sub>	394	136–139	0.43	85.6
19	C <sub>20</sub> H <sub>18</sub> N <sub>2</sub> O <sub>4</sub>	350	138–141	0.64	78.6
20	C <sub>21</sub> H <sub>20</sub> N <sub>2</sub> O <sub>4</sub>	364	166–169	0.49	77.8
21	C <sub>21</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub>	380	167–170	0.38	80.3
22	C <sub>20</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub> S	350	169–172	0.58	85.7
23	C <sub>20</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub>	328	196–199	0.47	87.5
24	C <sub>21</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub>	330	165–168	0.38	70.4
25	C <sub>23</sub> H <sub>18</sub> N <sub>2</sub> O <sub>3</sub>	370	157–160	0.71	80.0
26	C <sub>21</sub> H <sub>17</sub> N <sub>3</sub> O <sub>2</sub>	343	186–189	0.58	71.1
27	C <sub>20</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub>	318	180–183	0.57	72.0
28	C <sub>20</sub> H <sub>17</sub> ClN <sub>2</sub> O <sub>2</sub>	353	152–155	0.59	78.5
29	C <sub>20</sub> H <sub>17</sub> BrN <sub>2</sub> O <sub>2</sub>	397	166–169	0.62	79.9
30	C <sub>20</sub> H <sub>17</sub> N <sub>3</sub> O <sub>4</sub>	363	175–178	0.50	79.6
31	C <sub>20</sub> H <sub>17</sub> N <sub>3</sub> O <sub>4</sub>	363	162–165	0.45	75.7
32	C <sub>20</sub> H <sub>18</sub> N <sub>2</sub> O <sub>3</sub>	334	170–173	0.51	65.7
33	C <sub>20</sub> H <sub>18</sub> N <sub>2</sub> O <sub>3</sub>	334	211–214	0.59	72.5
34	C <sub>20</sub> H <sub>18</sub> N <sub>2</sub> O <sub>4</sub>	350	154–157	0.61	77.5
35	C <sub>20</sub> H <sub>17</sub> BrN <sub>2</sub> O <sub>2</sub>	397	182–185	0.68	80.5
36	C <sub>21</sub> H <sub>16</sub> BrN <sub>3</sub> O <sub>2</sub>	422	162–165	0.73	74.3

<sup>a</sup> TLC Mobile Phase—hexane:ethyl acetate 3:7

Further, the appearance of C–Br and C–F stretching bands at 510.10 and 1262.46 cm<sup>-1</sup> in compounds **7** and **8** showed the presence of bromo and fluoro groups in their structures, respectively. The presence of OH group in compounds **12**, **14**, and **35** was confirmed by C–O stretching band around 1250 cm<sup>-1</sup>. The skeletal C=C– stretching bands were observed in the range of 1200–1500 cm<sup>-1</sup> in the spectra of the synthesized compounds, which represents the

presence of aromatic rings. In compounds **17** and **19**, stretching bands in the region of 1160 cm<sup>-1</sup> (symmetric C–O–C stretching) and 1245 cm<sup>-1</sup> (asymmetric C–O–C stretching) revealed the presence of methoxy groups. Moreover, the aromatic nitro stretching around 1350 cm<sup>-1</sup> (symmetric NO<sub>2</sub> stretching) and 1540 cm<sup>-1</sup> (asymmetric NO<sub>2</sub> stretching) depicted the presence of nitro functional group in synthesized compounds **9**, **10**, **11**, and **32**.

#### Cytotoxic and antiviral activity

All the synthesized compounds were evaluated for antiviral activity and cytotoxicity. The inhibitory concentration of compounds was expressed as the concentration that caused 50% inhibition of viral cytopathogenicity (EC<sub>50</sub>). The cytotoxic concentration (CC<sub>50</sub>) of the compounds was monitored based on the growth of non-infected cells by the MTT method and corresponded to the compound concentration required to cause 50% cell death (Table 2) or on microscopically visible alteration of cell morphology (Tables 3, 4).

Generally, none of the compounds were inhibitory to HIV viruses tested at subtoxic concentrations (Table 2). Although in general most compounds were poorly cytotoxic to the MT-4 cell cultures (CC<sub>50</sub>: >50 µg/ml), several compounds showed pronounced cytotoxicity. In particular, compounds **12**, **20**, **25**, and **28** showed better cytotoxicity (CC<sub>50</sub>: 5.19, 6.23, 2.12, and 4.07 µg/ml) as compared to other synthesized derivatives, respectively.

It was interesting to notice that the position of the OH on the phenyl group is of crucial importance for eventual toxicity in the MT-4 cell cultures. The presence an OH in *ortho* position (**12**, **25**, and **34**) results in high toxicity (CC<sub>50</sub>: 5.19, 2.12, and 11.25 µg/ml), in *para* position (**14**) showed moderate toxicity (CC<sub>50</sub>: 26.50 µg/ml) and an OH at *meta* position (**13**) resulted in poor toxicity (CC<sub>50</sub>: 32.63 µg/ml) pointing to the free OH at *ortho* position as a prerequisite for cytotoxic activity. Further, the presence of both alkoxy and OH groups (compounds **19** and **20**) improved the cytotoxicity (CC<sub>50</sub>: 7.05 and 6.23 µg/ml). The compound synthesized from the *p*-chloroacetophenone (**28**) was also found to be highly toxic (CC<sub>50</sub>: 4.07 µg/ml) against selected HIV strains.

Similar type of results were also observed in case of varicella-zoster virus (VZV) cultured in human embryonic lung (HEL) cells, compounds having hydroxy groups particularly at *meta* and *para* position showed high toxicity, i.e., **13**, **14**, and **19** having EC<sub>50</sub> equal to or less than 20 µg/ml. Further, compound **28** was found to be highly cytotoxic (EC<sub>50</sub>: 2.50 µg/ml against TK<sup>+</sup> VZV strain; 3.24 µg/ml against TK<sup>-</sup> VZV strain and CC<sub>50</sub>: 7.1 µg/ml) synthesized from *p*-chloro acetophenone (Table 3). None of the compound was found to be active against cytomegalovirus (Table 4).

**Table 2** Cytostatic and anti-HIV activity of synthesized (naphthalen-1-yloxy)-acetic acid benzylidene/(1-phenyl-ethylidene)-hydrazide derivatives

Comp.	EC <sub>50</sub> <sup>a</sup> (μg/ml)		CC <sub>50</sub> <sup>b</sup> (μg/ml)
	HIV-1(IIIB)	HIV-2 (ROD)	
1	>96.60	>96.60	≥96.60
2	>56.20	>56.20	56.20 ± 1.52
3	>89.93	>89.93	89.93 ± 8.75
4	>87.60	>87.60	87.60 ± 7.67
5	>11.43	>11.43	11.43 ± 0.26
6	>87.43	>87.43	87.43 ± 15.44
7	>106.43	>106.43	106.43 ± 15.58
8	>65.05	>65.05	65.05 ± 12.10
9	>12.18	>12.18	12.18 ± 0.51
10	>12.10	>12.10	12.10 ± 0.10
11	>87.85	>87.85	87.85 ± 6.94
12	>5.19	>5.19	5.19 ± 3.15
13	>32.63	>32.63	32.63 ± 12.99
14	>26.50	>26.50	26.50 ± 6.90
15	>76.90	>76.90	76.90 ± 3.17
16	>19.25	>19.25	19.25 ± 2.59
17	>14.65	>14.65	14.65 ± 1.95
18	>13.40	>13.40	13.40 ± 1.11
19	>7.05	>7.05	7.05 ± 3.30
20	>6.23	>6.23	6.23 ± 3.15
21	>16.28	>16.28	16.28 ± 2.63
22	>68.33	>68.33	68.33 ± 19.77
23	>83.88	>83.88	83.88 ± 12.97
24	>62.68	>62.68	82.68 ± 28.47
25	>2.12	>2.12	2.12 ± 0.35
26	>51.53	>51.53	51.53 ± 22.51
27	>104.53	>104.53	104.53 ± 10.31
28	>4.07	>4.07	4.07 ± 3.78
29	>79.77	>79.77	79.77 ± 20.40
30	>77.60	>77.60	77.60 ± 5.11
31	>12.10	>12.10	12.10 ± 0.71
32	>12.08	>12.08	12.08 ± 1.29
33	>89.28	>89.28	89.28 ± 11.20
34	>11.25	>11.25	11.25 ± 0.66
35	>68.78	>68.78	68.78 ± 4.26
36	>59.90	>59.90	59.90 ± 2.60
Nevirapine	0.050 ± 0.011	>4.0	>4.0
Zidovudine	0.002 ± 0.001	0.0009 ± 0.0005	>25

<sup>a</sup> 50% Effective concentration or compound concentration required to inhibit HIV-induced cytopathicity in MT-4 cell cultures by 50%

<sup>b</sup> 50% Cytotoxic concentration or compound concentration required to reduce viability of the MT-4 cell cultures by 50% using the MTT methodology

**Table 3** Cytotoxicity and anti-virus activity of synthesized (naphthalen-1-yloxy)-acetic acid benzylidene/(1-phenyl-ethylidene)-hydrazide derivatives against varicella-zoster virus in human embryonic lung (HELL) cells

Comp.	EC <sub>50</sub> <sup>a</sup> (μg/ml)		CC <sub>50</sub> <sup>b</sup> (μg/ml)	
	TK <sup>+</sup> VZV strain	TK <sup>-</sup> VZV strain	(MCC) <sup>b</sup>	CC <sub>50</sub> <sup>c</sup> (μg/ml)
1	>20	>20	100	64.5
2	>20	>20	100	62.7
3	>100	>100	>100	58.6
4	>100	>100	>100	100
5	>4	>4	20	100
6	>20	>20	100	48.6
7	>100	>100	>100	100
8	>20	>20	100	71.6
9	>4	>4	20	100
10	>4	>4	20	61.2
11	>4	>4	20	14.2
12	>0.8	>0.8	4	3.57
13	9.2	16.2	100	62.7
14	9.6	10.5	≥20	60.6
15	>4	>4	20	100
16	>4	>20	≥20	56.9
17	>4	>20	≥20	52.6
18	12.5	17.4	100	47.9
19	13.8	20	100	27.7
20	>4	>4	20	18.1
21	16.9	>20	100	52.8
22	>20	>20	100	>100
23	>20	>20	100	80.1
24	>20	>20	100	100
25	>0.8	>0.8	4	1.94
26	>20	>20	100	>100
27	>20	>20	100	20
28	2.50	3.24	≥4	7.1
29	>4	>4	20	16.2
30	>4	>4	20	15.3
31	>4	>4	20	12.7
32	>4	>4	20	10.5
33	>20	>20	100	>100
34	7.0	7.9	100	4
35	>4	>0.8	≥4	11.1
36	58.5	>100	>100	74.8
Acyclovir	0.44	23	>100	120
Brivudin	0.0093	20	>100	173

<sup>a</sup> Effective concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU)

<sup>b</sup> Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology

<sup>c</sup> Cytotoxic concentration required to reduce cell growth by 50%

**Table 4** Cytotoxicity and anti-virus activity of synthesized (naphthalen-1-yloxy)-acetic acid benzylidene/(1-phenyl-ethylidene)-hydrazide derivatives against cytomegalovirus in human embryonic lung (HELL) cells

Comp.	EC <sub>50</sub> <sup>a</sup> (μg/ml)		CC <sub>50</sub> <sup>b</sup> (μg/ml)	
	AD-169 strain	Davis strain	(MCC) <sup>b</sup>	CC <sub>50</sub> <sup>c</sup> (μg/ml)
1	>20	>20	100	64.5
2	>20	>20	100	62.7
3	>20	>20	100	58.6
4	>20	>20	100	100
5	>4	>20	≥20	100
6	>20	>20	100	48.6
7	>100	>100	>100	100
8	>20	>20	100	71.6
9	>4	>4	20	100
10	>4	>20	≥20	61.2
11	>4	>4	20	14.2
12	>4	>4	20	3.57
13	>20	>20	100	62.7
14	>4	>4	20	60.6
15	>4	>4	20	100
16	>4	>20	≥20	56.9
17	>4	>20	≥20	52.6
18	>4	>20	≥20	47.9
19	>20	>20	100	27.7
20	>20	>20	100	18.1
21	>20	>20	100	52.8
22	>20	>20	≥100	>100
23	>4	>4	20	80.1
24	>20	>20	100	100
25	>0.8	>0.8	4	1.94
26	>20	>20	100	>100
27	>4	>4	20	20
28	>0.8	4	≥4	7.1
29	>4	>4	20	16.2
30	>4	>4	20	15.3
31	>4	>4	20	12.7
32	>4	>4	20	10.5
33	>20	>20	100	>100
34	>4	>4	20	4
35	>4	>4	20	11.1
36	>100	>100	>100	74.8
Ganciclovir	2.68	1.79	>100	60.5
Cidofovir	0.80	0.30	>100	35.4

<sup>a</sup> Effective concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU)

<sup>b</sup> Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology

<sup>c</sup> Cytotoxic concentration required to reduce cell growth by 50%

## Antibacterial and antifungal activity

The (naphthalen-1-yloxy)-acetic acid benzylidene/(1-phenyl-ethylidene)-hydrazide (**1–36**) derivatives were evaluated for their in vitro antibacterial activity against Gram-positive *Staphylococcus aureus*, *Bacillus subtilis*, Gram-negative *Escherichia coli* and antifungal activity against *Candida albicans* and *Aspergillus niger* by serial tube dilution method (Cappucino and Sherman, 1999) using norfloxacin and fluconazole as reference standards for antibacterial and antifungal activity, respectively, and the results are presented in Table 5.

Double strength nutrient broth I.P. and Sabouraud dextrose broth I.P. have been employed as media for growth of bacterial and fungal strains, respectively.

In case of *B. subtilis* compounds **18**, **19**, **20**, and **21** emerged as effective antibacterial agents having pMIC<sub>bs</sub> values 1.50, 1.45, 1.46, and 1.48, respectively. With regard to activity against *S. aureus*, the best activity was displayed by compounds **6**, **12**, **13**, **14**, **18**, and **32** having pMIC<sub>sa</sub> values more than 1.70. Whereas, analogues **18**, **31**, and **36** were found to be most active with pMIC<sub>ec</sub> values 1.50, 1.53, and 1.75, respectively, against *E. coli*. Among the tested bacterial strains, *S. aureus* showed relatively high sensitivity towards the tested compounds.

On the other hand, investigation of antifungal activity revealed that the compound **31** has shown marked inhibitory potential against *C. albicans* having pMIC<sub>ca</sub> value 2.13, which is comparable to standard drug fluconazole (pMIC<sub>ca</sub> = 2.64). For antifungal activity against *A. niger*, compounds **18**, **29**, and **31** have shown better antifungal potential having pMIC<sub>an</sub> values 1.50, 1.50, and 1.53, respectively, than the other synthesized derivatives. It can be seen from Table 5 that synthesized hydrazides have higher antifungal potential against *C. albicans* as compared to *A. niger*. Further, the results of antimicrobial activity revealed that compounds **18** and **31** displayed maximum inhibitory effect on the growth of tested bacterial and fungal strains as compared to other synthesized derivatives.

The obtained result revealed that the nature of substituents have a considerable impact on antibacterial and antifungal activities of target hydrazones and following structure–activity relationship (SAR) can be deduced.

## Structure–activity relationship

1. The analysis of antimicrobial studies indicated that the compounds having methoxy group (compounds **15–19**, **21**) except compound **15** were endowed with high antimicrobial activity against all the selected strains. This is supported by results of Emami *et al.* (2008).

**Table 5** Antimicrobial activity ( $\mu\text{m/ml}$ ) of synthesized (naphthalen-1-yloxy)-acetic acid benzylidene/(1-phenyl-ethylidene)-hydrazides

Comp.	pMIC <sub>bs</sub>	pMIC <sub>sa</sub>	pMIC <sub>ec</sub>	pMIC <sub>ca</sub>	pMIC <sub>an</sub>	pMIC <sub>b</sub>	pMIC <sub>f</sub>	pMIC <sub>am</sub>
<b>1</b>	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78
<b>2</b>	0.83	1.13	0.83	0.83	0.83	0.93	0.83	0.89
<b>3</b>	0.53	0.83	0.53	0.53	0.53	0.63	0.53	0.59
<b>4</b>	0.53	1.43	0.53	0.53	0.53	0.83	0.53	0.71
<b>5</b>	0.88	1.19	0.88	0.88	0.88	0.98	0.88	0.94
<b>6</b>	0.58	1.79	0.58	0.58	0.58	0.98	0.58	0.82
<b>7</b>	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58
<b>8</b>	1.11	1.41	1.41	0.81	0.81	1.31	0.81	1.11
<b>9</b>	1.14	1.45	1.45	1.45	1.45	1.35	1.45	1.39
<b>10</b>	1.14	1.14	1.45	1.45	0.84	1.24	1.15	1.20
<b>11</b>	1.14	0.84	0.84	1.45	0.54	0.94	1.00	0.96
<b>12</b>	1.11	1.71	1.41	1.41	1.11	1.41	1.26	1.35
<b>13</b>	1.11	1.71	1.41	1.41	1.41	1.41	1.41	1.41
<b>14</b>	1.11	1.71	1.41	1.71	1.11	1.41	1.41	1.41
<b>15<sup>a</sup></b>	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82
<b>16</b>	1.16	1.16	1.46	1.46	1.46	1.26	1.46	1.34
<b>17</b>	1.16	1.46	1.46	1.46	1.46	1.36	1.46	1.40
<b>18</b>	1.50	1.80	1.50	1.50	1.50	1.60	1.50	1.56
<b>19</b>	1.45	1.15	1.45	1.15	1.15	1.35	1.15	1.27
<b>20</b>	1.46	1.16	1.46	1.46	1.46	1.36	1.46	1.40
<b>21</b>	1.48	1.18	1.48	1.78	1.48	1.38	1.63	1.48
<b>22</b>	0.85	0.85	0.85	1.45	0.54	0.85	1.00	0.91
<b>23</b>	0.52	1.12	0.52	0.52	0.82	0.72	0.67	0.70
<b>24</b>	1.12	1.12	1.12	1.72	0.82	1.12	1.27	1.18
<b>25</b>	0.57	0.87	0.87	0.87	0.87	0.77	0.87	0.81
<b>26</b>	1.14	0.84	0.84	0.84	0.84	0.94	0.84	0.90
<b>27<sup>a</sup></b>	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82
<b>28</b>	1.15	1.45	1.15	1.75	0.85	1.25	1.30	1.27
<b>29<sup>a</sup></b>	1.20	1.20	1.20	1.50	1.50	1.20	1.50	1.32
<b>30<sup>a</sup></b>	0.90	0.60	0.60	0.60	0.60	0.70	0.60	0.66
<b>31</b>	1.23	1.23	1.53	2.13	1.53	1.33	1.83	1.53
<b>32</b>	1.16	1.76	1.46	1.76	1.46	1.46	1.61	1.52
<b>33</b>	1.16	1.16	1.46	1.46	0.86	1.26	1.16	1.22
<b>34<sup>a</sup></b>	0.82	1.13	1.43	0.82	0.82	1.13	0.82	1.00
<b>35<sup>a</sup></b>	1.13	1.13	1.43	1.43	1.43	1.23	1.43	1.31
<b>36<sup>a</sup></b>	1.15	1.15	1.75	1.45	1.45	1.35	1.45	1.39
SD	0.32	0.36	0.39	0.42	0.36	0.30	0.36	0.31
NAH	0.64	0.94	0.94	0.64	0.64	0.84	0.64	0.76
Std.	2.61 <sup>b</sup>	2.61 <sup>b</sup>	2.61 <sup>b</sup>	2.64 <sup>c</sup>	2.64 <sup>c</sup>	2.61	2.64	2.62

SD standard deviation, NAH 2-naphthoxy acetic acid hydrazide

<sup>a</sup> Outliers, <sup>b</sup> Norfloxacin,

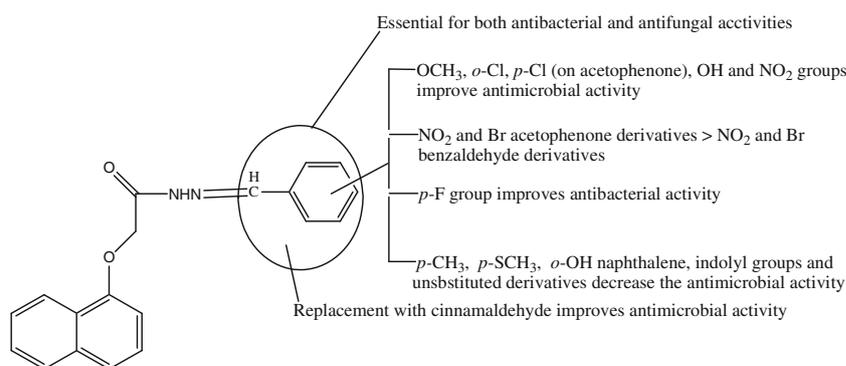
<sup>c</sup> Fluconazole

2. The results of antimicrobial activity showed that *ortho* substituted chloro derivative (compound **2**) has higher antimicrobial potency as compared to that of *meta* and *para* substituted (compounds **3** and **4**, respectively) chloro derivatives. This fact is supported by the observations of Guven *et al.* (2007). In contrary, compound **28** synthesized from *p*-chloro acetophenone has shown marked antimicrobial

potential against selected microorganisms except against *A. niger*.

3. It is noteworthy that the introduction of OH group (compounds **12–14** and **35–36**) gave new compounds with potent antimicrobial activity. This observation reveals the fact that presence of OH substituent increases the antimicrobial potential probably by forming hydrogen bond with the target biomacromolecule.

**Fig. 1** Structural requirements for the antimicrobial activity of synthesized hydrazone derivatives



This fact is supported by the observations of Vicini *et al.* (2002).

4. Compound **24** derived from cinnamaldehyde, has displayed better activity as compared to unsubstituted derivative (**1**). This may be due to the presence of extended conjugation in cinnamaldehyde portion and aromatic nucleus of 2-naphthoxy acetic acid, which may be involved in binding of synthesized compound with the target site. This fact is supported by one of our earlier studies (Narasimhan *et al.*, 2007).
5. The presence of electron withdrawing NO<sub>2</sub> group in compounds **9–11**, **32**, and **33** make them highly active antibacterial and antifungal agents. However, it is interesting to note that compounds synthesized from nitro acetophenones (**32** and **33**) were found to be more active than compounds synthesized from nitro benzaldehydes (**9–11**). The role of electron withdrawing group in increasing the antimicrobial activity is similar to the results of Sharma *et al.* (2004).
6. The presence of electron withdrawing fluorine group (compound **8**) in benzylidene moiety enhances the growth inhibition activity against bacterial strains as compared to unsubstituted analogue (compound **1**).
7. The substitution of bromine moiety in the synthesized analogues (compounds **5**, **6**, **7**, and **30**) lower the effectiveness against both bacteria and fungi. This behavior evidences that the introduction of bulky substituent significantly decrease the antimicrobial potency as mentioned by Zani *et al.* (2004). In contrary, in compounds **29** and **31** the presence of *o*-Br and *o*-Br-*m*-CN acetophenones resulted in marked improvement in antimicrobial activity particularly against fungal strains.
8. As regard the SARs, the introduction of thiomethyl (**22**), methyl (**23**), 2-hydroxyl naphthalene (**25**), and indolyl (**26**) moieties did not noticeably enhance growth inhibition potency of synthesized derivatives.
9. In general, the replacement of NH<sub>2</sub> group (compound NAH) with benzylidene/(1-phenyl-ethylidene) moieties (compounds **1–36**) led to noticeable increase

in antimicrobial activity of the synthesized compounds. This may be due to the increase in lipophilicity of the molecules which may allow them to easily penetrate the microbial membrane.

10. 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). The SAR results are summarized in Fig. 1.

## QSAR studies

### Development of one-target QSAR models (ot-QSAR)

In the present study, a classical Hansch approach using the linear free energy relationship (LFER) model described by Hansch and Fujita (1964) was employed to build the QSAR equations for identifying the substituent effect of synthesized compounds (**1–36**) against the five tested microbial strains.

Before using the antimicrobial activity data determined as MIC for QSAR study, they should be transformed to  $-\log$  MIC or pMIC in  $\mu\text{m/ml}$  (Table 5) to get all the values positive, normal distribution of errors and to get LFER of these data with physicochemical properties (Zhao *et al.*, 2007). Depending on the intercorrelation among the independent descriptors and also their individual correlation with antimicrobial activity, different possible combinations of parameters were subjected to LR and MLR analysis.

The different molecular parameters (independent variables), viz., thermodynamic, spatial, electronic, and topological parameters (Hansch and Fujita, 1964; Hansch *et al.*, 1973; Kier and Hall, 1973; Randic 1975; Balaban 1982; Wiener, 1947; Randic, 1993) were calculated using the molecular package TSAR 3.3 for windows (TSAR 3D Version 3.3, 2000) for substituted hydrazone derivatives are summarized in Table 6 and their respective values corresponding to their compounds are presented in Table 7.

**Table 6** Brief description of some molecular descriptors used in the present study

S. no.	Descriptor type	Molecular description
1	Topological	Kier's molecular connectivity ( ${}^0\chi$ , ${}^0\chi^v$ , ${}^1\chi$ , ${}^1\chi^v$ , ${}^2\chi$ , ${}^2\chi^v$ ), Kier's alpha shape ( $\kappa\alpha_1$ ) topological indices, Kier's shape ( $\kappa$ ) topological indices, Randic topological index ( $R$ ), Balaban topological index ( $J$ ), and Wiener topological index ( $W$ )
2	Quantum	Total energy (Te), Ionization potential (I.P.), energies of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), dipole moment ( $\mu$ ) and electronic energy (Ele.E)
3	Thermodynamic	$\log P$ (Octanol–water partition coefficient), Molar refractivity (MR)
4	Spatial	Molecular surface area, molar volume (Vm)
5	Structural	Molecular weight (MW)

Thermodynamic parameters describe free energy change during drug receptor complex formation. Spatial parameters are the quantified steric features of drug molecules required for its complimentary fit with receptor. Electronic parameters describe weak non-covalent bonding between drug molecules and receptor (Silakari *et al.*, 2008). Further, the topological descriptors are based on graph/structure concepts and geometric features such as shape, size, and branching (Lather and Madan, 2005). Units of the energy and dipole were electron volts (eV), and atomic units (a.u.), respectively (Dai *et al.*, 1999).

The substituents have to be used at least 3 times at the compounds for QSAR analysis. At the compounds **4**, **24**, **25**, and **26** there are some substituents used only once. So these compounds are not suitable for a QSAR work and so not included in QSAR studies. Further, it is noteworthy that no statistically significant equations were obtained for the remaining 32-data set as the value of regression coefficient ( $r$ ) is very less. The regression analysis of antimicrobial activity with different molecular parameters indicated that compounds **15**, **27**, **29**, **30**, **34**, **35**, and **36** gave high residues, i.e., the difference between observed and calculated pMIC. A compound was considered as an outlier for deriving a particular model when the residual value exceeded twice the standard error of estimate of the model (Prasanna *et al.*, 2005). Furthermore, in multivariate statistics, it is common to define following types of outliers (Furusjo *et al.*, 2006).

1. X outliers. Briefly, a substance is an X outlier if the molecular descriptors for this substance do not lie in the same range as the (rest of the) training data.
2. Y outliers are only defined for training or test samples. They are substances for which the reference value of response is invalid.

In view of above facts, a data set of 25 members, was generated for the model development and a marked improvement in the quality of correlations ( $r$  as high as 0.863) was observed.

The reference drugs norfloxacin and fluconazole were not included in model generation as they belong to

**Table 7** Value of QSAR descriptors of synthesized hydrazide derivatives used in regression analysis

Comp.	$\log P$	${}^0\chi$	${}^1\chi$	$\kappa_1$	$\kappa_2$	$B$	Nu.E.	HOMO
<b>1</b>	4.02	15.91	11.33	17.81	9.47	1.15	20701.50	-8.45
<b>2</b>	4.54	16.78	11.74	18.78	9.63	1.16	22712.80	-8.42
<b>3</b>	4.54	16.78	11.72	18.78	9.63	1.14	22151.20	-8.49
<b>4</b>	4.54	16.78	11.72	18.78	9.63	1.14	22148.30	-8.49
<b>5</b>	4.81	16.78	11.74	18.78	9.63	1.16	22652.80	-8.42
<b>6</b>	4.81	16.78	11.72	18.78	9.63	1.14	22092.90	-8.50
<b>7</b>	4.81	16.78	11.72	18.78	9.63	1.14	22090.30	-8.49
<b>8</b>	4.16	16.78	11.72	18.78	9.63	1.14	22279.60	-8.49
<b>9</b>	3.97	18.36	12.65	20.73	10.52	1.19	27696.10	-8.73
<b>10</b>	3.97	18.36	12.63	20.73	10.52	1.14	25218.80	-8.60
<b>11</b>	3.97	18.36	12.63	20.73	10.52	1.14	25219.60	-8.60
<b>12</b>	3.73	16.78	11.74	18.78	9.63	1.16	22881.70	-8.47
<b>13</b>	3.73	16.78	11.72	18.78	9.63	1.14	22316.70	-8.46
<b>14</b>	3.73	16.78	11.72	18.78	9.63	1.14	22312.20	-8.46
<b>15</b>	3.77	17.48	12.26	19.75	10.36	1.14	23945.90	-8.42
<b>16</b>	3.51	19.06	13.21	21.70	11.25	1.20	30088.30	-8.50
<b>17</b>	3.51	19.06	13.21	21.70	11.25	1.16	27780.60	-8.45
<b>18</b>	3.26	20.64	14.16	23.66	12.14	1.20	32430.60	-8.51
<b>19</b>	3.48	18.36	12.67	20.73	10.52	1.15	25897.90	-8.44
<b>20</b>	3.82	19.06	13.17	21.70	11.25	1.15	27597.00	-8.44
<b>21</b>	3.23	19.93	13.62	22.68	11.41	1.18	30287.30	-8.52
<b>22</b>	4.11	17.48	12.26	19.75	10.36	1.14	23705.90	-8.24
<b>23</b>	4.49	16.78	11.72	18.78	9.63	1.14	22269.90	-8.44
<b>24</b>	4.43	17.32	12.33	19.75	10.98	1.10	23046.00	-8.43
<b>25</b>	4.74	19.35	13.72	21.24	10.35	1.02	28469.50	-8.45
<b>26</b>	3.79	17.77	12.81	19.32	9.47	0.99	25455.10	-8.36
<b>27</b>	3.65	16.78	11.74	18.78	9.63	1.18	23987.30	-8.44
<b>28</b>	4.17	17.65	12.13	19.75	9.80	1.19	24239.30	-8.45
<b>29</b>	4.44	17.65	12.13	19.75	9.80	1.19	23679.90	-8.58
<b>30</b>	3.61	19.23	13.04	21.70	10.68	1.18	27147.60	-8.62
<b>31</b>	3.61	19.23	13.04	21.70	10.68	1.19	27475.40	-8.90
<b>32</b>	3.37	17.65	12.15	19.75	9.80	1.21	24946.90	-8.79
<b>33</b>	3.37	17.65	12.13	19.75	9.80	1.19	24546.60	-8.77
<b>34</b>	3.08	18.52	12.54	20.73	9.97	1.21	26786.00	-8.70
<b>35</b>	4.44	17.65	12.15	19.75	9.80	1.21	24761.60	-8.73
<b>36</b>	4.31	19.23	13.08	21.70	10.68	1.22	26179.90	-8.61

**Table 8** Correlation matrix for antibacterial activity of synthesizes hydrazides against *B. subtilis*

	log <i>P</i>	MR	${}^0\chi$	${}^0\chi^v$	$\kappa_1$	$\kappa\alpha_1$	<i>R</i>	T.E.	I.E.	LUMO	HOMO	pMIC <sub>bs</sub>
log <i>P</i>	1.000											
MR	−0.336	1.000										
${}^0\chi$	−0.660	0.861	1.000									
${}^0\chi^v$	−0.270	0.974	0.800	1.000								
$\kappa_1$	−0.655	0.868	0.999	0.805	1.000							
$\kappa\alpha_1$	−0.585	0.928	0.983	0.890	0.985	1.000						
<i>R</i>	−0.651	0.876	0.995	0.814	0.998	0.987	1.000					
T.E.	0.614	−0.802	−0.980	−0.752	−0.979	−0.957	−0.973	1.000				
I.E.	−0.366	−0.007	0.291	−0.024	0.262	0.171	0.217	−0.298	1.000			
LUMO	−0.141	0.020	−0.148	0.120	−0.135	−0.045	−0.105	0.227	−0.498	1.000		
HOMO	0.366	0.007	−0.291	0.024	−0.262	−0.171	−0.217	0.298	−1.000	0.498	1.000	
pMIC <sub>bs</sub>	−0.831	0.443	0.753	0.391	0.747	0.693	0.739	−0.766	0.260	−0.025	−0.260	1.000

**Table 9** Correlation of molecular descriptors with antimicrobial (antibacterial and antifungal) activity

	pMIC <sub>bs</sub>	pMIC <sub>sa</sub>	pMIC <sub>ec</sub>	pMIC <sub>ca</sub>	pMIC <sub>an</sub>	pMIC <sub>b</sub>	pMIC <sub>f</sub>	pMIC <sub>am</sub>
log <i>P</i>	−0.831	−0.348	−0.841	−0.766	−0.735	−0.786	−0.827	−0.838
MR	0.443	−0.015	0.242	0.385	0.399	0.248	0.431	0.346
${}^0\chi$	0.753	0.142	0.599	0.622	0.648	0.571	0.698	0.655
${}^0\chi^v$	0.391	0.030	0.196	0.302	0.369	0.229	0.365	0.304
${}^1\chi$	0.739	0.127	0.579	0.584	0.645	0.552	0.673	0.632
${}^1\chi^v$	0.232	−0.106	0.040	0.254	0.164	0.053	0.236	0.144
${}^2\chi$	0.743	0.134	0.603	0.662	0.610	0.566	0.704	0.654
${}^2\chi^v$	−0.064	−0.146	−0.229	0.049	−0.091	−0.180	−0.014	−0.107
$\kappa_1$	0.747	0.133	0.590	0.606	0.646	0.562	0.687	0.645
$\kappa\alpha_1$	0.693	0.119	0.516	0.547	0.601	0.505	0.629	0.585
$\kappa\alpha_2$	0.592	0.054	0.398	0.400	0.527	0.394	0.503	0.463
$\kappa\alpha_3$	0.435	−0.094	0.225	0.288	0.302	0.206	0.324	0.271
<i>R</i>	0.739	0.127	0.579	0.584	0.645	0.552	0.673	0.632
<i>B</i>	0.461	0.346	0.504	0.560	0.609	0.514	0.639	0.596
<i>W</i>	0.730	0.100	0.555	0.576	0.611	0.527	0.651	0.608
T.E.	−0.766	−0.155	−0.611	−0.573	−0.626	−0.586	−0.657	−0.643
LUMO	−0.025	0.188	0.023	−0.137	0.169	0.077	−0.003	0.044
HOMO	−0.260	−0.172	−0.398	−0.437	−0.354	−0.330	−0.439	−0.395

different structural series. A correlation analysis was performed on all the descriptors, depending on the inter-correlation among the independent descriptors and also their individual correlation with biological activity. A correlation matrix constructed for antibacterial activity against *B. subtilis* is presented in Table 8. The correlations of different molecular descriptors with antibacterial and antifungal activity are presented in Table 9. In general, high colinearity ( $r > 0.8$ ) was observed between different parameters. High interrelationship was observed between HOMO and I.E. ( $r = 1.000$ ),  ${}^1\chi$  and *R* ( $r = 0.995$ ) and low interrelationship was observed between MR and LUMO

( $r = 0.020$ ). Any combination of these descriptors in multiple regression analysis may result with model suffering from multi-colinearity. The correlation between antimicrobial activity and molecular descriptors (Table 9) indicated the predominance of partition coefficient (log *P*) in describing the antimicrobial activity of synthesized compounds.

In order to corroborate the validity of the derived QSAR models, LOO method was used (Schaper, 1999). In LOO method each compound is eliminated once, a model is derived from the remaining compounds and the eliminated compounds are predicted from this model. The same

**Table 10** Statistical parameters values calculated for the derived models for modeling antimicrobial activity of synthesized hydrazone derivatives

Eq. no.	$S_{\text{DEP}}$	PRESS	$S_{\text{PRESS}}$	SSY	$r^2$	RQR	$Q$	RMSE	$e^-$	VIF	PE	LSE	LOF	SEP
1	0.161	0.627	0.241	0.741	0.690	0.920	5.036	0.158	0.003	–	0.041	0.627	0.627	0.032
2	0.201	0.974	0.312	1.118	0.705	0.939	4.102	0.197	0.004	–	0.039	0.974	0.974	0.039
3	0.290	2.028	0.341	2.325	0.586	0.898	2.584	0.284	0.007	–	0.055	2.020	2.020	0.057
4	0.248	1.477	0.268	1.698	0.539	0.872	2.901	0.243	0.006	–	0.061	1.480	1.480	0.049
5	0.234	1.320	0.280	1.635	0.587	0.835	3.127	0.230	0.005	2.421	0.055	1.320	0.330	0.046
6	0.177	0.755	0.225	0.894	0.617	0.887	4.337	0.174	0.003	–	0.051	0.755	0.755	0.035
7	0.208	1.045	0.307	1.210	0.684	0.925	3.883	0.204	0.005	–	0.042	1.040	1.040	0.041
8	0.187	1.058	0.320	1.060	0.745	0.909	4.426	0.167	0.004	3.922	0.034	0.697	0.174	0.033
9	0.168	0.679	0.258	0.797	0.701	0.927	4.866	0.164	0.003	–	0.040	0.679	0.679	0.033

procedure is repeated after elimination of another compound until all the compounds have been eliminated once. Further, the statistical qualities of the developed models were judged by the parameters like correlation coefficient ( $r$ ), the cross-validated correlation coefficient ( $q^2$ ), standard error of estimate ( $s$ ), and Fischer's statistics ( $F$ ) at specified degrees of freedom. Sum of squared prediction errors called predictive residual sum of squares (PRESS) statistic also called as LSE is calculated as the sum of squares of the differences between predicted and observed values of the activity. Standard deviation of prediction ( $S_{\text{PRESS}}$ ), standard deviation of error of prediction ( $S_{\text{DEP}}$ ), absolute average error ( $e^-$ ), root mean square error (RMSE), quality value ( $Q$ ), square of correlation coefficient ( $r^2$ ), probable error of the coefficient of correlation (PE), Friedman's lack of fit measure (LOF), standard error of prediction (SEP), and sum of squares of response values (SSY) were calculated for each model and taken as an estimate of the predictability of the developed models (Table 10).

#### *ot*-QSAR model for antibacterial activity against *B. subtilis*

$$\text{pMIC}_{\text{bs}} = -0.488 \log P + 2.980$$

$$n = 25 \quad r = 0.831 \quad q^2 = 0.635 \quad s = 0.165 \quad F = 51.38 \quad (1)$$

For model 1, the cross-validated  $r^2$  ( $q^2$ ) and square of correlation coefficient ( $r^2$ ) are 0.635 and 0.690, which means that the resulted QSAR model could predict 63.5% and explain 69.0% of variances, respectively. Here,  $r^2$ , which presents the explained variance for given set, was also used to determine the goodness of model's fit performance. Eq. 1 also indicated statistical significance of >99.9% with  $F$  value of 51.38. The value of  $q^2$  obtained by LOO method is greater than 0.5 of Eq. 1 qualifies it to be a valid model according to recommendations of Golbraikh and Tropsha (2002).

As the coefficient of  $\log P$  in Eq. 1 is negative, therefore the antibacterial activity against *B. subtilis* will increase with decrease in value of  $\log P$ . This is clearly, evident from Table 7 that compounds 18, 19, 20, and 21 having low  $\log P$  values, 3.26, 3.48, 3.82, and 3.23, have high antibacterial activity values 1.50, 1.45, 1.46, and 1.48 (Table 5), respectively. Similarly, compounds 3 and 23 having high  $\log P$  values, i.e., 4.54 and 4.49 (Table 7), have minimum antibacterial activity values, i.e., 0.53 and 0.52, respectively, against *B. subtilis*. Similarly, Eqs. 2, 3, and 4 demonstrated that antimicrobial potential of *E. coli*, *C. albicans*, and *A. niger* is negatively correlated with  $\log P$ , respectively. The negative coefficient of  $\log P$  indicated that an increase in the  $\log P$  of molecule hinders the ligand to pass through cell membrane and thus decrease in activity.

Progress in the use of QSAR methods has shown the importance of the hydrophobic or lipophilic nature of biologically active molecules. The lipophilicity modifies the penetration of bioactive molecules through the apolar cell membranes. This property is usually characterized by the partition coefficient ( $\log P$ ), which is essentially determined from distribution studies of the compound between an immiscible polar and non-polar solvent pair. This quantitative descriptor of lipophilicity ( $\log P$ ) is one of the key determinants of pharmacokinetic properties. Knowing the exact values for this parameter, it is possible to predict the inhibitory activity of the drugs (Podunavac-Kuzmanovic *et al.*, 2008a).

$\log P$  is the logarithm of the ratio of the concentrations of the un-ionized solute in two solvents, which is calculated according to following equation, where o is octanol and w is un-ionized water.

$$\log P_{\text{o/w}} = \log\left(\frac{[\text{solute}]_{\text{o}}}{[\text{solute}]_{\text{w}}}\right)$$

The hydrophobic effect is the major driving force for the binding of drugs to their receptor targets in

pharmacodynamics, and is based on the log  $P$  contribution of each atom. Each atom in a molecule contributes to the log  $P$  by the amount of its atomic parameter multiplied by the degree of exposure to the surrounding solvent (Park *et al.*, 2008).

To systematically assess a prediction algorithm, a reliable validation is required. Usually, a predictive method is evaluated by the predictive results for a training data set and testing data set, respectively. According to the statistical terminology, the former is called a test of resubstitution, and the latter is a test of cross-validation. By the test of resubstitution, the value of each compound in a training data set is predicted using the rules derived from the same set. This test gives somewhat optimistic error estimate because the same compounds are used to derive the prediction rules and to predict themselves. On the other hand, a cross-validation test for an independent testing data set is also needed because it can reflect the generalizing effectiveness of a predictive method. Of various cross-validation tests, the LOO test is thought to be a reliable one. By the LOO test, the statistical parameter of the derived model is

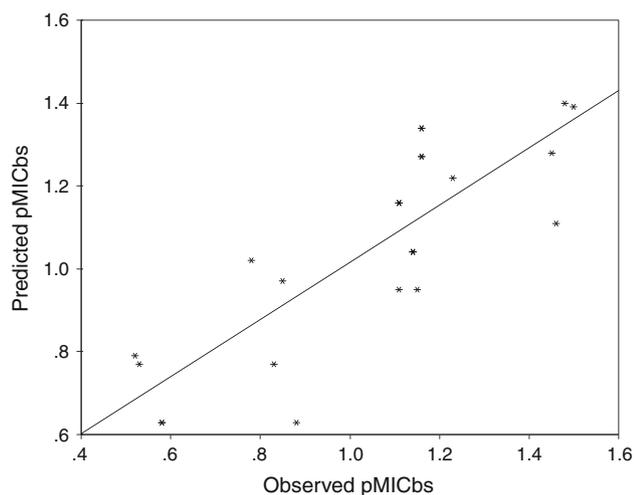
predicted by the rules derived from all the other compounds except the predicted one. In other words, in the LOO test each compound is singled out as an independent sample and used to examine the predictive method. Both tests of resubstitution and LOO were used to evaluate the prediction of models developed in the current study. In the resubstitution test, three statistical parameters, viz., regression coefficient ( $r$ ), root mean square error (RMSE), and absolute average error ( $e^-$ ) were used (Table 10) to evaluate the performance (Zhao *et al.*, 2007).

In addition, the prediction performance of the built models must be estimated to build a successful QSAR model. The comparison of observed and predicted antibacterial activities is presented in Table 11. It can be seen from the results that the observed and predicted antibacterial activities lie close to each other as evidenced by their low residual values. Further, the plot of LR predicted  $\text{pMIC}_{\text{bs}}$  against observed  $\text{pMIC}_{\text{bs}}$  (Fig. 2) also favors the model expressed by Eq. 1.

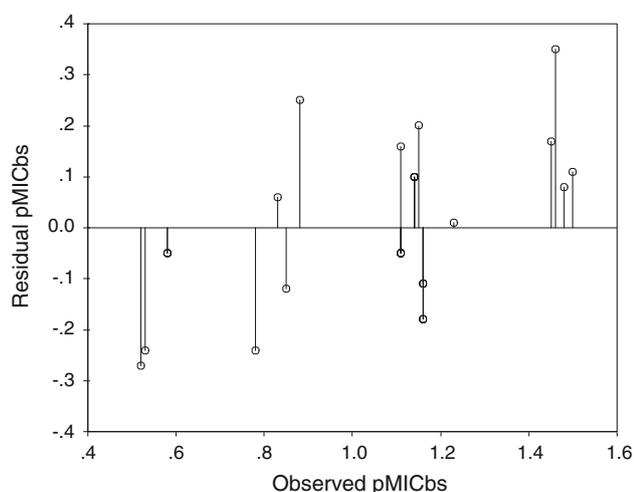
To investigate the existence of a systemic error in developing the QSAR model, the residuals  $\text{pMIC}_{\text{bs}}$  values

**Table 11** Comparison of observed and predicted antibacterial and antifungal activity obtained by ot-QSAR models

Comp.	$\text{pMIC}_{\text{bs}}$ using Eq. 1			$\text{pMIC}_{\text{ec}}$ using Eq. 2			$\text{pMIC}_{\text{ca}}$ using Eq. 3			$\text{pMIC}_{\text{an}}$ using Eq. 5		
	Obs.	Pre.	Res.									
1	0.78	1.02	-0.24	0.78	1.13	-0.35	0.78	1.22	-0.44	0.78	0.87	-0.09
2	0.83	0.77	0.06	0.83	0.80	0.03	0.83	0.86	-0.03	0.83	0.72	0.11
3	0.53	0.77	-0.24	0.53	0.80	-0.27	0.53	0.86	-0.33	0.53	0.72	-0.19
5	0.88	0.63	0.25	0.88	0.63	0.25	0.88	0.67	0.21	0.88	0.61	0.27
6	0.58	0.63	-0.05	0.58	0.63	-0.05	0.58	0.67	-0.09	0.58	0.61	-0.03
7	0.58	0.63	-0.05	0.58	0.63	-0.05	0.58	0.67	-0.09	0.58	0.61	-0.03
8	1.11	0.95	0.16	1.41	1.04	0.37	0.81	1.12	-0.31	0.81	0.87	-0.06
9	1.14	1.04	0.10	1.45	1.16	0.29	1.45	1.25	0.20	1.45	1.08	0.37
10	1.14	1.04	0.10	1.45	1.16	0.29	1.45	1.25	0.20	0.84	1.07	-0.23
11	1.14	1.04	0.10	0.84	1.16	-0.32	1.45	1.25	0.20	0.54	1.07	-0.53
12	1.11	1.16	-0.05	1.41	1.31	0.10	1.41	1.42	-0.01	1.11	1.04	0.07
13	1.11	1.16	-0.05	1.41	1.31	0.10	1.41	1.42	-0.01	1.41	1.04	0.37
14	1.11	1.16	-0.05	1.41	1.31	0.10	1.71	1.42	0.29	1.11	1.04	0.07
16	1.16	1.27	-0.11	1.46	1.45	0.01	1.46	1.58	-0.12	1.46	1.34	0.12
17	1.16	1.27	-0.11	1.46	1.45	0.01	1.46	1.58	-0.12	1.46	1.34	0.12
18	1.50	1.39	0.11	1.50	1.61	-0.11	1.50	1.75	-0.25	1.50	1.58	-0.08
19	1.45	1.28	0.17	1.45	1.47	-0.02	1.15	1.60	-0.45	1.15	1.28	-0.13
20	1.46	1.11	0.35	1.46	1.25	0.21	1.46	1.36	0.10	1.46	1.21	0.25
21	1.48	1.40	0.08	1.48	1.63	-0.15	1.78	1.77	0.01	1.48	1.52	-0.04
22	0.85	0.97	-0.12	0.85	1.07	-0.22	1.45	1.16	0.29	0.54	0.97	-0.43
23	0.52	0.79	-0.27	0.52	0.83	-0.31	0.52	0.89	-0.37	0.82	0.74	0.08
28	1.15	0.95	0.20	1.15	1.03	0.12	1.75	1.12	0.63	0.85	0.92	-0.07
31	1.23	1.22	0.01	1.53	1.39	0.14	2.13	1.51	0.62	1.53	1.28	0.25
32	1.16	1.34	-0.18	1.46	1.54	-0.08	1.76	1.68	0.08	1.46	1.25	0.21
33	1.16	1.34	-0.18	1.46	1.54	-0.08	1.46	1.68	-0.22	0.86	1.25	-0.39



**Fig. 2** Plot of observed  $\text{pMIC}_{\text{bs}}$  versus predicted  $\text{pMIC}_{\text{bs}}$  for LR model by Eq. 1



**Fig. 3** Plot of observed  $\text{pMIC}_{\text{bs}}$  versus residual  $\text{pMIC}_{\text{bs}}$  for LR model by Eq. 1

were plotted against the observed  $\text{pMIC}_{\text{bs}}$  values in Fig. 3. The propagation of the residual values on both sides of zero indicates that no systemic error exists in the development of LR model (Jalali-Heravi and Kyani, 2004).

*ot*-QSAR model for antibacterial activity against *E. coli*

$$\begin{aligned} \text{pMIC}_{\text{cc}} &= -0.631 \log P + 3.66 \\ n = 25 \quad r &= 0.841 \quad q^2 = 0.662 \quad s = 0.205 \quad F = 55.21 \end{aligned} \quad (2)$$

For model 2 the value of  $r^2$  and  $q^2$  is 0.705 (Table 10) and 0.662, which means the QSAR model is able explain 70.5% and predict 66.2% variances of the antimicrobial activity, respectively. Further, the coefficient of  $\log P$  in Eq. 2 is negative, indicating that the antibacterial activity of

synthesized derivatives against *E. coli* is inversely proportional to the value of  $\log P$ . This is evidenced by the antibacterial activity data of substituted hydrazides (Table 5) and their  $\log P$  values (Table 7). Compounds **18** and **21** with low  $\log P$  values of 3.26 and 3.23 (Table 7) have maximum antibacterial activity against *E. coli* (compound **18**,  $\text{pMIC}_{\text{cc}} = 1.50$ ; compound **21**,  $\text{pMIC}_{\text{cc}} = 1.48$ , Table 5). Similarly, the compounds **3** and **23** with high  $\log P$  (Table 7) values have minimum antibacterial activity ( $\text{pMIC}_{\text{cc}} = 0.53$  and 0.52, Table 5). In the case of *S. aureus* the regression analysis did not yield valid *ot*-QSAR model.

*ot*-QSAR model for antifungal activity against *C. albicans*

$$\begin{aligned} \text{pMIC}_{\text{ca}} &= -0.699 \log P + 4.034 \\ n = 25 \quad r &= 0.765 \quad q^2 = 0.526 \quad s = 0.296 \quad F = 32.61 \end{aligned} \quad (3)$$

*ot*-QSAR model for antifungal activity against *A. niger*

$$\begin{aligned} \text{pMIC}_{\text{an}} &= -0.543 \log P + 3.185 \\ n = 25 \quad r &= 0.734 \quad q^2 = 0.501 \quad s = 0.253 \quad F = 26.96 \end{aligned} \quad (4)$$

The QSAR models elicited in Eqs. 3 and 4 once again indicated the importance of  $\log P$  ( $r = 0.766$  and 0.734) in demonstrating antifungal activity of substituted benzylidene hydrazide derivatives against *C. albicans* and *A. niger*, respectively.

$$\begin{aligned} \text{pMIC}_{\text{an}} &= -0.403 \log P + 0.143^1 \chi + 0.861 \\ n = 25 \quad r &= 0.766 \quad q^2 = 0.490 \quad s = 0.245 \quad F = 15.67 \end{aligned} \quad (5)$$

In MLR analysis, the coefficient of regression ( $r$ ) increases to 0.766 (Eq. 5) from 0.734 (Eq. 4, in LR) on addition of  $^1\chi$  to  $\log P$ , thus leading to an increase in predictability of *mt*-QSAR models, which can be observed from low residual values (Table 11). Similar to Eq. 1 the high  $q^2$  values ( $q^2 > 0.5$ ) obtained by LOO technique and observance of low residual values (Table 11) indicated the validity and predictability of Eqs. 2, 3, and 4.

The cross-validated correlation coefficient ( $r_{\text{cv}}^2 > 0.5$ ) values obtained for the best QSAR models indicated their reliability in predicting the antimicrobial activity of substituted hydrazide derivatives. In case of *A. niger* the  $r_{\text{cv}}^2$  ( $q^2$ ) value is equal to 0.5, which shows that the developed model is an invalid one. But one should not forget the recommendations of Golbraikh and Tropsha (2002) 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

*A. niger* (Eq. 4) is a valid one. Moreover, PRESS is a good estimate of the real prediction error of the model. Its value less than SSY indicate that the proposed models have good predictive power (Kumar *et al.*, 2006). Thus, in view of this, all the models proposed by us (Eqs. 1–5) are statistically significant which can be easily clarified by their values listed in Table 10. The internal consistency of the developed models using  $q^2$  can also assessed with the ratio  $RQR = q^2/r^2$  (Sivaprakasam *et al.*, 2006). Considering RQR for the QSAR models represented by Eqs. 1–5 (Table 10), it is pleasing that the lowest value is  $RQR = 0.835$  for QSAR model 5 and there is a gradual, roughly monotonic increase across the series to  $RQR = 0.939$ . Thus, the QSAR models are statistically robust.

Generally for QSAR studies, the biological activities of compounds should span 2–3 orders of magnitude. But in the present study the range of activities of the compounds is within one order of magnitude. But it is important to note that the predictability of the QSAR models developed in the present study is very high as evidenced by the low residual values (Table 11). 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. Further, recent literature reveals that the QSAR has been applied to describe the relationship between narrow range of biological activity and physicochemical properties of the molecules (Narasimhan *et al.*, 2007; Sharma *et al.*, 2006; Hatya *et al.*, 2006; Kumar *et al.*, 2006). 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 (Narasimhan *et al.*, 2007, Kumar *et al.*, 2007). Further, according to recommendation of Kim *et al.* (2007) if the standard deviation  $s$  is not much larger than 0.3, the models are acceptable. Hence, the minimum standard deviation (Table 5) observed in the antimicrobial activity data justifies its use in QSAR studies.

Further, the low values of PE, LSE, LOF, SEP, SSY,  $S_{\text{PRESS}}$ ,  $S_{\text{DEP}}$ , RMSE, and  $e^-$  and high values of  $Q$ ,  $r^2$ , and  $q^2$  (Table 10) revealed the statistical significance of the model described by Eqs. 1–5. In case of *S. aureus* statistical significant model can not be developed as the value of regression coefficient ( $r$ ) is very less (Table 9).

#### Development of multi-target QSAR (mt-QSAR) model

According to the above ot-QSAR models one should use five different equations with different errors to predict the activity of a new compound against the five microbial species. The ot-QSAR models, which are almost in all the literature, become unpractical or at less complicated to use when we have to predict to each compound results for more than one target. In these cases we have to develop one ot-QSAR for

each target. However, very recently the interest has been increased in development of multi-target QSAR (mt-QSAR) models. In opposition 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 (Prado-Prado *et al.*, 2008; Gonzalez-Diaz *et al.*, 2007, 2008; Gonzalez-Diaz and Prado-Prado, 2008; Cruz-Monteagudo *et al.*, 2007).

In the present study we have 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 substituted benzylidene hydrazides against all the above mentioned microorganisms.

In order to develop mt-QSAR models, initially we have calculated the average antibacterial activity, antifungal activity, and antimicrobial activity values of substituted hydrazide derivatives which are presented in Table 5. These average activity values were also correlated with the molecular descriptors of synthesized compounds (Table 9).

The mt-QSAR model for antibacterial activity revealed the importance of partition coefficient ( $\log P$ ) in describing the antibacterial activity of substituted benzylidene hydrazides (Eq. 6).

#### mt-QSAR model for antibacterial activity

$$\begin{aligned} \text{pMIC}_b &= -0.455 \log P + 2.964 \\ n = 25 \quad r &= 0.785 \quad q^2 = 0.547 \quad s = 0.181 \quad F = 37.08 \end{aligned} \quad (6)$$

It is worth to mention here that in case of ot-QSAR, developed model of *S. aureus* can not accurately predict antibacterial activity as the regression analysis did not yield valid QSAR model ( $r = 0.348$ ), whereas in case of mt-QSAR, developed model (Eq. 6,  $r = 0.785$ ) are valid and can be used to predict the antibacterial activity including *S. aureus* of synthesized hydrazide derivatives.

#### mt-QSAR model for antifungal activity

$$\begin{aligned} \text{pMIC}_f &= -0.621 \log P + 3.600 \\ n = 25 \quad r &= 0.827 \quad q^2 = 0.633 \quad s = 0.213 \quad F = 49.85 \end{aligned} \quad (7)$$

$$\begin{aligned} \text{pMIC}_t &= -0.591 \log P - 0.554J - 2.280 \\ n = 25 \quad r &= 0.852 \quad q^2 = 0.623 \quad s = 0.195 \\ F &= 28.03 \end{aligned} \quad (8)$$

The mt-QSAR model for antifungal activity signifies the importance of partition coefficient ( $\log P$ ) in describing antifungal activity. Further, the coefficient of  $\log P$  in the Eq. 7 is negative, indicating that the antifungal activity of synthesized derivatives is inversely proportional to the value of  $\log P$ . This is evidenced by the antifungal activity data of substituted hydrazides (Table 5) and their  $\log P$  values (Table 7). The value of  $q^2$  is 0.633 which means that the developed model could predict 63.3% variance of antifungal activity. Further, during MLR study, there is slight increase in value of regression coefficient ( $r = 0.827$ , Eq. 7 to  $r = 0.852$ , Eq. 8), hence Eq. 8 is the most predictive one for describing the antifungal potential of synthesized hydrazide derivatives.

Topological indices (viz., Balaban index ( $J$ )) are numerical parameters of a graph which characterize its topology and are usually graph invariant. Topological indices are used in the development of QSARs in which the biological activity or other properties of molecules are correlated with their chemical structure. Topological descriptors are derived from hydrogen-suppressed molecular graphs, in which the atoms are represented by vertices and the bonds by edges. The connections between the atoms can be described by various types of topological matrices (e.g., distance or adjacency matrices), which can be mathematically manipulated so as to derive a single number, usually known as graph invariant, graph-theoretical index, or topological index (Gonzalez-Diaz *et al.*, 2008).

#### mt-QSAR models for antimicrobial activity

$$\text{pMIC}_{\text{am}} = -0.520 \log P + 3.220$$

$$n = 25 \quad r = 0.837 \quad q^2 = 0.650 \quad s = 0.172 \quad (9)$$

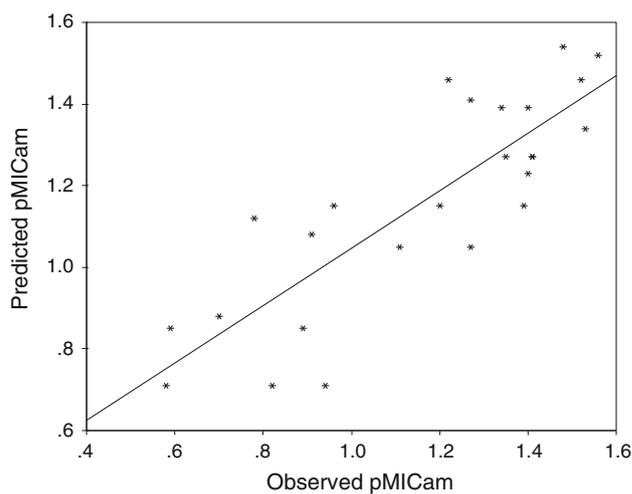
$$F = 54.09$$

Similarly, as in aforementioned QSAR studies, the developed mt-QSAR model Eq. 9 revealed the importance of  $\log P$  in demonstrating the overall antimicrobial activity of synthesized derivatives. This is evidenced by antimicrobial activity data (Table 5) and their  $\log P$  values (Table 7).

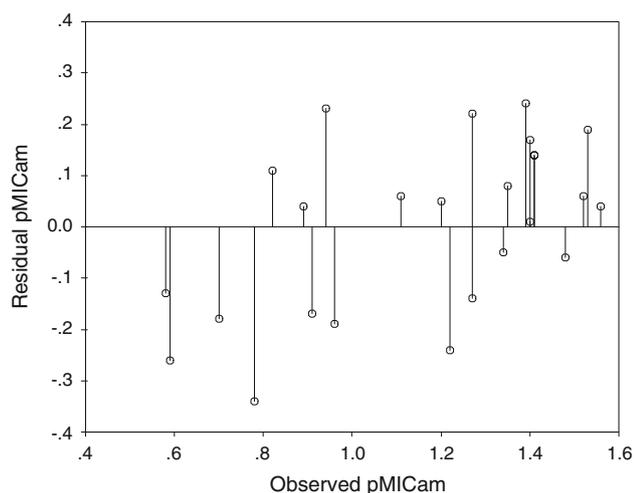
In order to confirm our results we have predicted the antibacterial, antimicrobial, and antifungal activity values using mt-QSAR Eqs. 6, 8, and 9 (Table 12). The comparison of observed and predicted values (Table 12) demonstrated that they are close to each other, evidenced by their low residual values. Further, the results are supported by plot of observed  $\text{pMIC}_{\text{am}}$  and predicted  $\text{pMIC}_{\text{am}}$  (Fig. 4). To investigate the existence of a systemic error in developing the QSAR model, the residuals  $\text{pMIC}_{\text{am}}$  values were plotted against the observed  $\text{pMIC}_{\text{am}}$  values in Fig. 5. The propagation of the residual values on both sides of zero

**Table 12** Comparison of observed and predicted antibacterial and antifungal activity obtained by mt-QSAR models

Comp.	$\text{pMIC}_b$ using Eq. 6			$\text{pMIC}_f$ using Eq. 8			$\text{pMIC}_{\text{am}}$ using Eq. 9		
	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.
1	0.78	1.13	-0.35	0.78	1.04	-0.26	0.78	1.12	-0.34
2	0.93	0.90	0.03	0.83	0.87	-0.04	0.89	0.85	0.04
3	0.63	0.90	-0.27	0.53	0.76	-0.23	0.59	0.85	-0.26
5	0.98	0.77	0.21	0.88	0.73	0.15	0.94	0.71	0.23
6	0.98	0.77	0.21	0.58	0.62	-0.04	0.82	0.71	0.11
7	0.58	0.77	-0.19	0.58	0.63	-0.05	0.58	0.71	-0.13
8	1.31	1.07	0.24	0.81	0.97	-0.16	1.11	1.05	0.06
9	1.35	1.15	0.20	1.45	1.30	0.15	1.39	1.15	0.24
10	1.24	1.15	0.09	1.15	1.03	0.12	1.20	1.15	0.05
11	0.94	1.15	-0.21	1.00	1.06	-0.06	0.96	1.15	-0.19
12	1.41	1.26	0.15	1.26	1.28	-0.02	1.35	1.27	0.08
13	1.41	1.26	0.15	1.41	1.17	0.24	1.41	1.27	0.14
14	1.41	1.26	0.15	1.41	1.18	0.23	1.41	1.27	0.14
16	1.26	1.36	-0.10	1.46	1.54	-0.08	1.34	1.39	-0.05
17	1.36	1.36	0.00	1.46	1.36	0.10	1.40	1.39	0.01
18	1.60	1.48	0.12	1.50	1.67	-0.17	1.56	1.52	0.04
19	1.35	1.38	-0.03	1.15	1.33	-0.18	1.27	1.41	-0.14
20	1.36	1.22	0.14	1.46	1.15	0.31	1.40	1.23	0.17
21	1.38	1.49	-0.11	1.63	1.61	0.02	1.48	1.54	-0.06
22	0.85	1.09	-0.24	1.00	0.97	0.03	0.91	1.08	-0.17
23	0.72	0.92	-0.20	0.67	0.80	-0.13	0.70	0.88	-0.18
28	1.25	1.06	0.19	1.30	1.16	0.14	1.27	1.05	0.22
31	1.33	1.32	0.01	1.83	1.46	0.37	1.53	1.34	0.19
32	1.46	1.43	0.03	1.61	1.66	-0.05	1.52	1.46	0.06
33	1.26	1.43	-0.17	1.16	1.56	-0.40	1.22	1.46	-0.24



**Fig. 4** Plot of observed  $\text{pMIC}_{\text{am}}$  versus predicted  $\text{pMIC}_{\text{am}}$  for LR model by Eq. 9



**Fig. 5** Plot of observed  $pMIC_{am}$  versus residual  $pMIC_{am}$  for LR model by Eq. 9

indicates that no systemic error exists in the development of LR model.

The non-collinearity between the descriptors was tested through variance inflation factor (VIF) and tolerance. In practice, when  $VIF > 5$  or higher, or if the tolerance remains under the value 0.20, then this would indicate that there exists multicollinearity among the descriptors. Table 10 shows that there is no significant correlation between the selected descriptors ( $VIF < 5$ ); hence, there is no multicollinearity problem on the selected subset of molecular descriptors which indicated that MLR models expressed by Eqs. 5 and 8 (Table 10) are statistically valid (Goodarzi *et al.*, 2010).

Further, the statistical quality of the developed models (Eqs. 6–9) was gaged by the low values of parameters like PE, LSE, LOF, SEP, SSY,  $S_{press}$ ,  $S_{DEP}$ , RMSE, and  $e^-$  and high value of quality value  $Q$ ,  $r^2$ , and  $q^2$  (Table 10).

In general from the above-mentioned ot- and mt-QSAR models, it can be concluded that strong influence of the partition coefficient,  $\log P$ , is important for the antimicrobial activity of synthesized hydrazide derivatives. This evidence was clearly described in lipid theory advanced by Meyer and Overton (Podunavac-Kuzmanovic *et al.*, 2008b). According to this theory,  $\log P$  is a measure of hydrophobicity, which is important for the penetration and distribution of the drug, but also for the interaction of the drug with receptors. Therefore, it can be suggested that lipophilic properties have to be checked for designing of potent antimicrobial agents as they are decisive factors for their activity.

## Conclusion

A series of (naphthalen-1-yloxy)-acetic acid benzylidene/(1-phenyl-ethylidene)-hydrazides (**1–36**) have been

synthesized successfully in appreciable yields and screened for their in vitro antiviral (against HIV-I, HIV-II, VZV, and cytomegalovirus strains) and antimicrobial activities against bacterial strains *S. aureus*, *B. subtilis*, *E. coli*, and fungal strains *C. albicans* and *A. niger*. The antiviral evaluation depicted that none of the synthesized derivatives inhibited the replication of tested virus strains at subtoxic concentrations. However, the presence of OH group improved the cytotoxic activity against HIV (at *ortho* position) and varicella-zoster virus (at *meta* and *para* position) as compared to other synthesized derivatives. The SAR studies indicated that the introduction of 3,4,5-trimethoxy benzaldehyde (**18**), *o*-Br, *p*-CN (**31**), and *m*-NO<sub>2</sub> acetophenone (**32**) substituents improved the antimicrobial activity of synthesized hydrazide derivatives. Moreover, to understand the relationship between physicochemical parameters and antimicrobial activity of substituted hydrazide derivatives, QSAR investigation was performed by development of ot- and mt-QSAR models. The multi-target QSAR models were found to be more effective in describing the antimicrobial activity of substituted hydrazides than the one-target models. Further, it revealed the importance of the partition coefficient ( $\log P$ ) in describing the antimicrobial activity of substituted hydrazides. The validity of models obtained by linear and MLRs are clearly evidenced by the high  $q^2$  values and the low residual activity obtained for the developed QSAR models.

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