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ORIGINAL PAPER

N′-[4-[(Substituted imino)methyl]benzylidene]-substituted benzohydrazides: synthesis, antimicrobial, antiviral, and anticancer evaluation, and QSAR studies

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Abstract A variety of *N′*-[4-[(substituted imino)methyl]-benzylidene]-substituted benzohydrazides have been synthesized and evaluated for antimicrobial and anticancer potential. Results from testing of antimicrobial activity indicated the most potent antimicrobial agents had pMIC_{am} = 1.51. The synthesized compounds were bacteriostatic and fungistatic in action. Results from evaluation of antiviral activity indicated that none of the synthesized hydrazide derivatives inhibited viral replication at subtoxic concentrations. Results from anti-HIV screening against HIV-2 strain ROD indicated that one compound was more potent ($IC_{50} \geq 1$ μg/cm^3) than the standard drug nevirapine ($IC_{50} \geq 4$ μg/cm^3) and another was equipotent ($IC_{50} \geq 4$ μg/cm^3). The most effective anticancer agent against both HCT116 and MCF7 cancer cell lines had $IC_{50} = 19$ and 18 μg/cm^3, respectively. QSAR analysis indicated the importance of Wiener index (*W*) and energy of the lowest unoccupied molecular orbital (LUMO) in describing the antimicrobial activity of the synthesized compounds.

Keywords Hydrazides · Antimicrobial · Antiviral · Anticancer · QSAR

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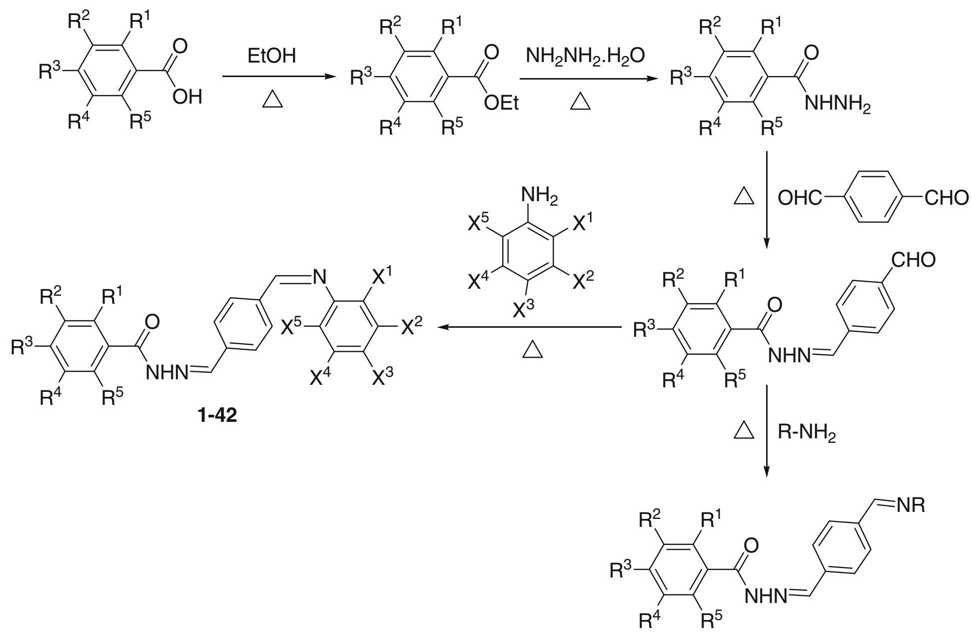
Introduction

The dramatically rising prevalence of multi-drug-resistant microbial infections in the past few decades has become a serious health care problem. In particular, the emergence of multi-drug resistant strains of Gram-positive bacterial pathogens, for example methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermis* and vancomycin-resistant *Enterococcus* is a problem of ever-increasing significance. One way of overcoming this challenge is control of the use of currently marketed antibiotics; another is the development of novel antimicrobial agents. Consequently, the search for new antimicrobial agents will remain an important and challenging task for medicinal chemists [1].

Acquired immune deficiency syndrome (AIDS) has become a global pandemic and has claimed more lives than any other disease. According to the 2008 UNAIDS report released by the WHO, 33 million people were diagnosed as human immunodeficiency virus (HIV)-positive in 2007 [2]. Human T lymphocytes are target cells for HIV replication, and the MT-4 cell line is used for the screening of anti-HIV agents. In recent years, much attention has been devoted to the search for effective chemotherapeutic agents for inhibition of HIV with minimum side effects [3].

Cancer is a disease of worldwide importance. Its incidence in the developed countries is increasing, and its

Scheme 1



mortality is ranked second in the order of causes of death. A similar tendency is observed in the developing world: gradual improvement in life expectancy is associated with elevated cancer incidence and mortality. Malignancy and its consecutive burden is therefore a global problem resulting in widespread interest in cancer therapy [4].

The synthesis of novel pharmacologically active molecules with reduced toxicity is of prime interest. Recently, quantitative structure–activity relationships (QSAR) have gained importance in medicinal chemistry. QSAR are predictive tools used for preliminary evaluation of the activity of chemical compounds by use of computer-aided models. Use of QSAR has increased the probability of success in the drug-discovery process and reduced the time and cost involved [5].

Hydrazones have attracted the attention of many chemists owing to their wide range of biological activity. Literature reports reveal that chemistry and biology of hydrazones have been intensively investigated during the past decade. Hydrazone derivatives have been found to have antimicrobial [6], anticancer [7], antimycobacterial [8], antimalarial [9], antioxidant [10], anticonvulsant [11], analgesic [12], anti-inflammatory [13], and antidiabetic [14] activity.

Motivated by these facts, and in continuation of our research efforts in synthesis, evaluation of antimicrobial activity, and QSAR studies [15–18], we report herein the synthesis, antimicrobial, antiviral, and anticancer evaluation, and QSAR studies of *N*′-[4-[(substituted imino)methyl]benzylidene]-substituted benzohydrazides.

Results and discussion

Chemistry

Synthesis of the target compounds (**1–48**) was performed as outlined in Scheme 1. All the compounds were high-melting-point solids (m.p. > 300 °C). The structures of the compounds **1–48** were ascertained on the basis of their consistent IR, NMR, and mass spectral characteristics and results from elemental (C, H, N) analysis, all of which were in full agreement with their assigned molecular structures.

Antimicrobial activity

The synthesized compounds were screened for their in-vitro antibacterial activity against *S. aureus*, *Bacillus subtilis*, and *Escherichia coli* and antifungal activity against *Candida albicans* and *Aspergillus niger* by the tube dilution method [19] using norfloxacin and fluconazole as reference standards for antibacterial and antifungal activity, respectively; the results are presented in Table 1.

Among the synthesized compounds, *N*′-[4-[(2-chloro-4-nitrophenylimino)methyl]benzylidene]-4-chlorobenzohydrazide (**6**), *N*′-[4-[(2-chloro-4-nitrophenylimino)methyl]benzylidene]-4-aminobenzohydrazide (**33**), and *N*’-[4-[(2-chloro-4-nitrophenylimino)methyl]benzylidene]-4-hydroxybenzohydrazide (**37**) were found to be effective against *S. aureus*, having pMIC_{sa} values of 1.25, 1.23, and 1.23, respectively. Against *B. subtilis*, *E. coli*, and *C. albicans*, compounds **6**, **33**, and **37** emerged as the most effective antimicrobial agents with p*MIC* values of 1.55, 1.53, and

Table 1 Antimicrobial activity (p*MIC*/μM cm^{-3}) of N′-[4-[(substituted imino)methyl]benzylidene]-substituted benzohydrazides

Comp.	p*MIC*sa	p*MIC*bs	p*MIC*ec	p*MIC*ca	p*MIC*an	p*MIC*ab	p*MIC*af	p*MIC*am
1	1.16	1.16	1.46	1.46	1.46	1.26	1.46	1.34
2	1.20	1.20	1.50	1.50	1.50	1.30	1.50	1.38
3	1.19	1.49	1.49	1.49	1.49	1.39	1.49	1.43
4	1.19	1.19	1.49	1.49	1.49	1.29	1.49	1.37
5	1.19	1.49	1.49	1.49	1.49	1.39	1.49	1.43
6	1.25	1.55	1.55	1.55	1.55	1.45	1.55	1.49
7	1.21	1.51	1.51	1.51	1.51	1.41	1.51	1.45
8	1.21	1.21	1.51	1.51	1.51	1.31	1.51	1.39
9	1.14	1.14	1.44	1.44	1.44	1.24	1.44	1.32
10	1.18	1.18	1.48	1.48	1.48	1.28	1.48	1.36
11	1.19	1.19	1.49	1.49	1.49	1.29	1.49	1.37
12	1.17	1.17	1.47	1.47	1.47	1.27	1.47	1.35
13	1.14	1.14	1.44	1.44	1.44	1.24	1.44	1.32
14	1.18	1.18	1.48	1.48	1.48	1.28	1.48	1.36
15	1.17	1.17	1.47	1.47	1.47	1.27	1.47	1.35
16	1.17	1.17	1.47	1.47	1.47	1.27	1.47	1.35
17	1.13	1.13	1.44	1.44	1.44	1.24	1.44	1.32
18	1.18	1.48	1.48	1.48	1.48	1.38	1.48	1.42
19	1.17	1.47	1.47	1.47	1.47	1.37	1.47	1.41
20	1.18	1.48	1.48	1.48	1.48	1.38	1.48	1.42
21	1.14	1.44	1.44	1.44	1.44	1.34	1.44	1.38
22	1.18	1.48	1.48	1.48	1.48	1.38	1.48	1.42
23	1.17	1.47	1.47	1.47	1.47	1.37	1.47	1.41
24	1.21	1.51	1.51	1.51	1.51	1.41	1.51	1.45
25	1.21	1.51	1.51	1.51	1.81	1.41	1.66	1.51
26	1.17	1.47	1.47	1.47	1.77	1.37	1.62	1.47
27	1.21	1.51	1.51	1.51	1.81	1.41	1.66	1.51
28	1.19	1.49	1.49	1.49	1.79	1.39	1.64	1.49
29	1.18	1.18	1.48	1.48	1.78	1.28	1.63	1.42
30	1.19	1.19	1.49	1.49	1.79	1.29	1.64	1.43
31	1.19	1.49	1.49	1.49	1.79	1.39	1.64	1.49
32	1.21	1.21	1.51	1.51	1.51	1.31	1.51	1.39
33	1.23	1.53	1.53	1.53	1.53	1.43	1.53	1.47
34	1.18	1.48	1.48	1.48	1.48	1.38	1.48	1.42
35	1.19	1.49	1.49	1.49	1.19	1.39	1.34	1.37
36	1.19	1.49	1.49	1.49	1.49	1.39	1.49	1.43
37	1.23	1.53	1.53	1.53	1.53	1.43	1.53	1.47
38	1.19	1.49	1.49	1.49	1.49	1.39	1.49	1.43
39	1.18	1.48	1.18	1.48	1.48	1.28	1.48	1.36
40	1.18	1.48	1.48	1.48	1.48	1.38	1.48	1.42
41	1.16	1.46	1.46	1.46	1.16	1.36	1.31	1.34
42	1.20	1.50	1.50	1.50	1.50	1.40	1.50	1.44
43	1.11	1.41	1.41	1.41	1.41	1.31	1.41	1.35
44	1.15	1.45	1.45	1.45	1.45	1.35	1.45	1.39
45	1.09	1.09	1.09	1.39	1.39	1.09	1.39	1.21
46	1.05	1.35	1.35	1.35	1.05	1.25	1.20	1.23
47	1.20	1.50	1.50	1.50	1.50	1.40	1.50	1.44
48	1.09	1.39	1.39	1.39	1.39	1.29	1.39	1.33
SD	0.04	0.15	0.08	0.04	0.15	0.07	0.09	0.06
Std.	2.61*	2.61*	2.61*	2.64**	2.64**	–	–	–

* Norfloxacin

** Fluconazole

Table 2 Minimum bactericidal/fungicidal concentrations of *N*′-[4-[(substituted imino)methyl]benzylidene]-substituted benzohydrazides

Comp.	Minimum bactericidal/fungicidal concentration/μM cm^{-3}				
	S. aureus	*B. subtilis*	*E. coli*	*C. albicans*	*A. niger*
1	>0.14	>0.14	>0.14	>0.14	0.14
2	>0.13	>0.13	>0.13	>0.13	>0.13
3	>0.13	>0.13	>0.13	>0.13	>0.13
4	>0.13	0.13	>0.13	>0.13	>0.13
5	>0.13	>0.13	>0.13	>0.13	>0.13
6	>0.11	>0.11	>0.11	>0.11	>0.11
7	>0.12	>0.12	>0.12	>0.12	0.12
8	>0.12	0.12	>0.12	>0.12	>0.12
9	>0.15	>0.15	>0.15	0.15	>0.15
10	0.13	0.13	>0.13	>0.13	0.13
11	0.13	0.13	>0.13	>0.13	>0.13
12	>0.13	>0.13	>0.13	0.13	>0.13
13	>0.15	>0.15	>0.15	>0.15	>0.15
14	>0.13	>0.13	>0.13	>0.13	0.07
15	0.14	0.14	>0.14	>0.14	>0.14
16	>0.14	>0.14	>0.14	>0.14	>0.14
17	>0.15	>0.15	>0.15	0.15	>0.15
18	>0.13	>0.13	>0.13	>0.13	>0.13
19	>0.14	>0.14	>0.14	>0.14	>0.14
20	0.13	>0.13	>0.13	>0.13	>0.13
21	0.15	>0.15	>0.15	>0.15	>0.14
22	0.13	>0.13	>0.13	>0.13	>0.13
23	0.13	>0.13	>0.13	>0.13	>0.13
24	>0.12	>0.12	>0.12	>0.12	>0.12
26	>0.13	>0.13	>0.13	>0.13	>0.13
27	>0.12	>0.12	>0.12	>0.12	0.06
28	>0.13	>0.13	>0.13	>0.13	>0.13
29	>0.13	>0.13	>0.13	>0.13	>0.13
30	>0.13	0.13	>0.13	>0.13	0.13
31	>0.13	>0.13	>0.13	>0.13	>0.13
32	>0.12	>0.12	>0.12	>0.12	>0.12
33	>0.12	>0.12	>0.12	>0.12	>0.12
34	>0.13	>0.13	>0.13	>0.13	>0.13
35	>0.13	>0.13	>0.13	>0.13	>0.13
36	>0.13	>0.13	>0.13	>0.13	>0.13
37	>0.12	>0.12	>0.12	>0.12	0.12
38	>0.13	>0.13	>0.13	>0.13	0.13
39	>0.13	>0.13	>0.13	>0.13	>0.13
40	>0.13	>0.13	>0.13	>0.13	>0.13
41	>0.14	>0.14	>0.14	>0.14	>0.14
42	>0.13	>0.13	>0.13	>0.13	>0.13
43	>0.16	>0.16	>0.16	>0.16	>0.16
44	>0.14	>0.14	>0.14	>0.14	>0.14
45	>0.16	>0.16	>0.16	>0.16	>0.16
46	>0.18	>0.18	>0.18	>0.18	>0.18
47	>0.13	>0.13	>0.13	>0.13	>0.13
48	0.16	0.16	>0.16	>0.16	>0.16
Standard	0.019[a]	0.019[a]	0.019[a]	0.040[b]	0.040[b]

[a] Norfloxacin

[b] Fluconazole

Table 3 Anti-feline corona virus (FIPV) and anti-feline herpes virus activity and cytotoxicity of the synthesized hydrazide derivatives in CRFK cell cultures

Comp.	CC_{50} [a]/μg cm^{-3}	EC_{50} [b]/μg cm^{-3}		Comp.	CC_{50} [a]/μg cm^{-3}	EC_{50} [b]/μg cm^{-3}	
		Feline corona virus	Feline herpes virus			Feline corona virus	Feline herpes virus
1	>100	>100	>100	**26**	>100	>100	>100
2	>100	>100	>100	**27**	>100	>100	>100
3	>100	>100	>100	**28**	>100	>100	>100
4	>100	>100	>100	**29**	>100	>100	>100
5	>100	>100	>100	**30**	>100	>100	>100
6	>100	>100	>100	**31**	>100	>100	>100
7	>100	>100	>100	**32**	>100	>100	>100
8	>100	>100	>100	**33**	>100	>100	>100
9	>100	>100	>100	**34**	>100	>100	>100
10	>100	>100	>100	**35**	>100	>100	>100
11	>100	>100	>100	**36**	>100	>100	>100
12	>100	>100	>100	**37**	>100	>100	>100
13	34.1	>20	>20	**38**	>100	>100	>100
14	>100	>100	>100	**39**	>100	>100	>100
15	>100	>100	>100	**40**	>100	>100	>100
16	>100	>100	>100	**41**	>100	>100	>100
17	>100	>100	>100	**42**	>100	>100	>100
18	>100	>100	>100	**43**	>100	>100	>100
19	>100	>100	>100	**44**	>100	>100	>100
20	>100	>100	>100	**45**	>100	>100	>100
21	>100	>100	>100	**46**	>100	>100	>100
22	>100	>100	>100	**47**	>100	>100	>100
23	>100	>100	>100	**48**	>100	>100	>100
24	>100	>100	>100	HHA	>100	7.6	1.1
25	>100	>100	>100	UDA	43.7	1.8	0.8
				Ganciclovir (μM)	>100	>100	0.7

CRFK cells Crandell–Rees feline kidney cells

[a] 50 % Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay

[b] 50 % Effective concentration, or concentration resulting in 50 % inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay

1.53, respectively (Table 1). N′-[4-[(4-Chlorophenylimino)methyl]benzylidene]-3-nitrobenzohydrazide (**25**) and N′-[4-[(2-chlorophenylimino)methyl]benzylidene]-4-nitrobenzohydrazide (**27**) were found to be effective against *A. niger* with a p*MIC* value of 1.81 (for both **25** and **27**) and proved the most effective antimicrobial agents with a pMIC_{am} value of 1.51.

In general, results from minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) studies (Table 2) revealed that the synthesized compounds were bacteriostatic and fungistatic in action, because their MFC and MBC values were threefold higher than their MIC values (a drug is considered to be bacteriostatic/fungistatic when its MFC and MBC values are threefold higher than its MIC value) [20].

Antiviral activity

The antiviral assays were based on inhibition of virus-induced cytopathicity in CRFK, HEL, Vero, HeLa, and MT-4 cell cultures. The results of the antiviral evaluation are given in Tables 3, 4, 5 and 6. None of the synthesized hydrazide derivatives inhibited viral replication at subtoxic concentrations.

Anti-HIV activity

The anti-HIV activity and cytotoxicity were evaluated against HIV-1 strain IIIB and HIV-2 strain ROD in MT-4 cell cultures using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [21]; the

Table 4 Cytotoxicity and antiviral activity of the synthesized hydrazide derivatives in HEL cell cultures

Comp.	Minimum cytotoxic concentration[a]/μg cm^{-3}	EC_{50}[b]/μg cm^{-3}				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK-KOS ACV[r]
1	>100	>100	>100	>100	>100	>100
2	100	>20	>20	>20	>20	>20
3	>100	>100	>100	>100	>100	>100
4	>100	>100	>100	>100	>100	>100
5	>100	>100	>100	>100	>100	>100
6	>100	>100	>100	>100	>100	>100
7	>100	>100	>100	>100	>100	>100
8	>100	>100	>100	>100	>100	>100
9	100	>20	>20	>20	>20	>20
10	100	>20	>20	>20	>20	>20
11	100	>20	>20	>20	>20	>20
12	>100	>100	>100	>100	>100	>100
13	>100	>100	>100	>100	>100	>100
14	>100	>100	>100	>100	>100	>100
15	>100	>100	>100	>100	>100	>100
16	100	>20	>20	>20	>20	>20
17	>100	>100	>100	>100	>100	>100
18	>100	>100	>100	>100	>100	>100
19	>100	>100	>100	>100	>100	>100
20	>100	>100	>100	>100	>100	>100
21	>100	>100	>100	>100	>100	>100
22	>100	>100	>100	>100	>100	>100
23	>100	>100	>100	>100	>100	>100
24	>100	>100	>100	>100	>100	>100
25	>100	>100	>100	>100	>100	>100
26	>100	>100	>100	>100	>100	>100
27	>100	>100	>100	>100	>100	>100
28	>100	>100	>100	>100	>100	>100
29	>100	>100	>100	>100	>100	>100
30	>100	>100	>100	>100	>100	>100
31	>100	>100	>100	>100	>100	>100
32	>100	>100	>100	>100	>100	>100
33	>100	>100	>100	>100	>100	>100
34	>100	>100	>100	>100	>100	>100
35	>100	>100	>100	>100	>100	>100
36	>100	>100	>100	>100	>100	>100
37	>100	>100	>100	>100	>100	>100
38	>100	>100	>100	>100	>100	>100
39	>100	>100	>100	>100	>100	>100
40	>100	>100	>100	>100	>100	>100
41	>100	>100	>100	>100	>100	>100
42	>100	>100	>100	>100	>100	>100
43	>100	>100	>100	>100	>100	>100
44	>100	>100	>100	>100	>100	>100
45	>100	>100	>100	>100	>100	>100
46	>100	>100	>100	>100	>100	>100

Table 4 continued

Comp.	Minimum cytotoxic concentration[a]/μg cm^{-3}	EC_{50}[b]/μg cm^{-3}				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK-KOS ACV^r
47	100	>20	>20	>20	>20	>20
48	>100	>100	>100	>100	>100	>100
Brivudin (μM)	>250	0.03	96	29	>250	250
Cidofovir (μM)	>250	1.2	1.2	10	>250	2
Acyclovir (μM)	>250	0.4	0.4	>250	>250	183
Ganciclovir (μM)	>100	0.05	0.08	>100	>100	20

[a] Amount required to cause a microscopically detectable alteration of normal cell morphology

[b] Amount required to reduce virus-induced cytopathogenicity by 50 %

results are presented in Table 7. The results indicated that compound **29** ($IC_{50} \geq 1$ μg/cm^3) was more potent than the standard drug nevirapine ($IC_{50} \geq 4$ μg/cm^3) against the HIV-2 strain ROD; compound **39** ($IC_{50} \geq 4$ μg/cm^3) was found to be equipotent.

Anticancer activity

The anticancer activity of the synthesized hydrazide derivatives against human colon cancer (HCT116) and breast cancer (MCF7) cell lines was determined by use of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay [22]; the results are presented in Table 8.

In general, the synthesized compounds had poor anticancer potential. Compounds **13**, **20**, **22**, and **48** were effective against HCT116 cancer cell lines with IC_{50} values of 40, 19, 30, and 30 μg/cm^3, respectively, and compounds **15**, **16**, and **20** were effective against MCF7 cancer cell lines with IC_{50} values of 35, 25, and 18 μg/cm^3, respectively.

The results from antimicrobial, antiviral, and anticancer evaluation revealed that the nature of the substituents has a substantial effect on the biological activity of the target hydrazones; the structure–activity relationships (SAR) discussed below were deduced.

Structure–activity relationships

Results of antimicrobial screening indicated that presence of electron-withdrawing 2-chloro-4-nitro substituents on the phenylimino structure of compounds **6**, **33**, and **37** increases antimicrobial activity against *S. aureus*, *B. subtilis*, *E. coli*, and *C. albicans*, whereas the presence of an electron-withdrawing nitro group on the benzoic acid structure and a chloro group on the phenylimino structure increases antifungal activity against *A. niger*. It is important to note that the incubation temperature was the same for all three bacterial species and *C. albicans* (a fungus). These similar incubation conditions may be the reason for the higher activity of compounds **6**, **33**, and **37** against *C. albicans* and the bacterial species. The effect of the electron-withdrawing group in improving antimicrobial activity is supported by the studies of Sharma et al. [23].

1. The presence of an electron-releasing group on the benzoic acid structure (**13**, **29**, and **39**) increases the anti-HIV activity of the synthesized compounds.
2. The presence of an electron-releasing group on the benzoic acid structure (**13**, **15**, **16**, **20**, and **22**) increases the anticancer potential of the synthesized compounds. The effect of an electron-releasing group in improving the anticancer potential of benzodiazepine derivatives is similar to the observation of Kamal et al. [24].
3. The presence of an imino moiety does not improve the antimicrobial and anticancer potential of the synthesized compounds except for the propylimino moiety (**48**) which improved anticancer activity against human colon cancer cell lines (HCT 116).
4. The presence of the naphthalene-1-ylimino moiety (**47**) in the synthesized compounds does not improve antimicrobial and anticancer potential.
5. The aforementioned results indicate that different structural requirements are essential for a compound to be selected as antibacterial or antifungal agent. This is similar to results obtained by Sortino et al. [25].

QSAR studies

To identify the effects of substituents on antimicrobial activity, quantitative structure–activity relationship (QSAR)

Table 5 Cytotoxicity and antiviral activity of the synthesized hydrazide derivatives in HeLa cell cultures

Comp.	Minimum cytotoxic concentration[a]/ μg cm^{-3}	EC_{50}[b]/μg cm^{-3}		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
1	100	>20	>20	>20
2	100	>20	>20	>20
3	≥20	>20	>20	>20
4	≥20	>20	>20	>20
5	≥20	>20	>20	>20
6	≥20	>20	>20	>20
7	≥20	>20	>20	>20
8	≥20	>20	>20	>20
9	≥20	>20	>20	>20
10	≥20	>20	>20	>20
11	≥20	>20	>20	>20
12	≥20	>20	>20	>20
13	100	>20	>20	>20
14	100	>20	>20	>20
15	>100	>100	>100	>100
16	>100	>100	>100	>100
17	>100	>100	>100	>100
18	≥20	>20	>20	>20
19	>100	>100	>100	>100
20	>100	>100	>100	>100
21	>100	>100	>100	>100
22	>100	>100	>100	>100
23	>100	>100	>100	>100
24	>100	>100	>100	>100
25	>100	>100	>100	>100
26	>100	>100	>100	>100
27	>100	>100	>100	>100
28	>100	>100	>100	>100
29	>100	>100	>100	>100
30	>50	>50	>50	>50
31	>100	>100	>100	>100
33	>100	>100	>100	>100
34	>100	>100	>100	>100
35	>100	>100	>100	>100
36	>100	>100	>100	>100
37	>100	>100	>100	>100
38	>100	>100	>100	>100
39	>100	>100	>100	>100
40	>100	>100	>100	>100
41	>100	>100	>100	>100
42	>100	>100	>100	>100
43	>100	>100	>100	>100
44	>100	>100	>100	>100
45	>100	>100	>100	>100
46	>100	>100	>100	>100
47	>100	>100	>100	>100
48	>100	>100	>100	>100
DS-5000	>100	20	34	4
(*S*)-DHPA (μM)	>250	>250	>250	>250
Ribavirin (μM)	>250	4	22	10

[a] Amount required to cause a microscopically detectable alteration of normal cell morphology

[b] Amount required to reduce virus-induced cytopathogenicity by 50 %

studies were undertaken, using the linear free energy relationship (LFER) model described by Hansch and Fujita [26]. In this study, all synthesized *N*′-[4-[(substituted imino)methyl]benzylidene]-substituted benzohydrazides (**1–48**) were used for linear regression model generation.

The standard drugs norfloxacin and fluconazole were not used for model development because they differ in structure from the synthesized compounds. Biological activity data determined as MIC values were first transformed into p*MIC* values (i.e. −log MIC, Table 1); these were used as dependent variables in the QSAR study. The different molecular descriptors (independent variables), for example the logarithm of the octanol–water partition coefficient (log *P*), molar refractivity (MR), Kier's molecular connectivity ($^0\chi$, $^0\chi^v$, $^1\chi$, $^1\chi^v$, $^2\chi$, $^2\chi^v$) and shape (κ_1, $\kappa\alpha_1$, $\kappa\alpha_2$, $\kappa\alpha_3$) topological indices, the Randic topological index (*R*), the Balaban topological index (*J*), the Wiener topological index (*W*), total energy (*Te*), energies of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), dipole moment (μ) and electronic energy (Ele. E) [26–31] were calculated for the hydrazide derivatives. Some of the values obtained are presented in Table 9.

Our earlier studies [15–17] indicated that multi-target QSAR (mt-QSAR) models are more suitable than one-target QSAR (ot-QSAR) models for describing antimicrobial activity. In this study, therefore, we developed multi-target QSAR models to describe the antimicrobial activity of compounds **1–48**.

For ot-QSAR models one should use five different equations with different errors to predict the activity of a new compound against five microbial species. Use of ot-QSAR models, which are used almost throughout the literature, were not practical, however, when we had to predict each compound's action against more than one target. In those cases we had to develop one ot-QSAR model for each target.

Table 6 Cytotoxicity and antiviral activity of the synthesized hydrazide derivatives in Vero cell cultures

Comp.	Minimum cytotoxic concentration[a]/μg cm^{-3}	EC_{50}[b]/μg cm^{-3}				
		Para-influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
1	>100	>100	>100	>100	>100	>100
2	>100	>100	>100	>100	>100	>100
3	>100	>100	>100	>100	>100	>100
4	>100	>100	>100	>100	>100	>100
5	100	>20	>20	>20	>20	>20
6	100	>20	>20	>20	>20	>20
7	>100	>100	>100	>100	>100	>100
8	100	>20	>20	>20	>20	>20
9	≥20	>20	>20	>20	>20	>20
10	100	>20	>20	>20	>20	>20
11	100	>20	>20	>20	>20	>20
12	100	>20	>20	>20	>20	>20
13	100	>20	>20	>20	>20	>20
14	100	>20	>20	>20	>20	>20
15	>100	>100	>100	>100	>100	>100
16	>100	>100	>100	>100	>100	>100
17	>100	>100	>100	>100	>100	>100
18	>100	>100	>100	>100	>100	>100
19	>100	>100	>100	>100	>100	>100
20	>100	>100	>100	>100	>100	>100
21	>100	>100	>100	>100	>100	>100
22	>100	>100	>100	>100	>100	>100
23	>100	>100	>100	>100	>100	>100
24	>100	>100	>100	>100	>100	>100
25	>100	>100	>100	>100	>100	>100
26	>100	>100	>100	>100	>100	>100
27	>100	>100	>100	>100	>100	>100
28	>100	>100	>100	>100	>100	>100
29	>100	>100	>100	>100	>100	>100
30	>100	>100	>100	>100	>100	>100
31	>100	>100	>100	>100	>100	>100
32	>100	>100	>100	>100	>100	>100
33	>100	>100	>100	>100	>100	>100
34	>100	>100	>100	>100	>100	>100
35	>100	>100	>100	>100	>100	>100
36	>100	>100	>100	>100	>100	>100
37	>100	>100	>100	>100	>100	>100
38	>100	>100	>100	>100	>100	>100
39	100	>20	>20	>20	>20	>20
40	100	>20	>20	>20	>20	>20
41	100	>20	>20	>20	>20	>20
42	>100	>100	>100	>100	>100	>100
43	>100	>100	>100	>100	>100	>100
44	>100	>100	>100	>100	>100	>100
45	>100	>100	>100	>100	>100	>100
46	>100	>100	>100	>100	>100	>100

Table 6 continued

Comp.	Minimum cytotoxic concentration[a]/μg cm^{-3}	EC_{50}[b]/μg cm^{-3}				
		Para-influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
47	100	>20	>20	>20	>20	>20
48	100	>20	>20	>20	>20	>20
DS-5000	>100	>100	>100	20	96	100
(*S*)-DHPA (μM)	>250	>250	>250	>250	>250	>250
Ribavirin (μM)	>250	50	>250	>250	>250	50

[a] Amount required to cause a microscopically detectable alteration of normal cell morphology

[b] Amount required to reduce virus-induced cytopathogenicity by 50 %

However, very recently interest has 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 [32–35].

In this study, we attempted to develop three different types of mt-QSAR model, viz. an mt-QSAR model to describe the antibacterial activity of the synthesized compounds against *S. aureus*, *B. subtilis*, and *E. coli*, an mt-QSAR model to describe the antifungal activity of the synthesized compounds against *C. albicans* and *A. niger*, and a common mt-QSAR model to describe the antimicrobial (overall antibacterial and antifungal) activity of the synthesized hydrazide derivatives against all the above mentioned microorganisms.

To develop mt-QSAR models, initially we calculated the average antibacterial, antifungal, and antimicrobial activity of the hydrazide derivatives (Table 1). These average antibacterial activity values were correlated with the molecular descriptors of the synthesized compounds (Table 10).

A high interrelationship was observed among topological indices, the Wiener index (W), and the Randic index (R, $r = 0.989$), and a low interrelationship was observed between the lipophilic parameter log P and the electronic property energy of the highest occupied molecular orbital (HOMO) ($r = 0.046$). Correlation of average antibacterial, antifungal, and antimicrobial activity values with different molecular descriptors is shown in Table 11.

From the correlation matrix (Table 10), it was observed that the electronic property total energy (Te) dominated description of the antibacterial activity of the synthesized compounds (Eq. 1).

LR-mt-QSAR model for antibacterial activity:

$$\mathrm{p}MIC_{\mathrm{ab}} = -0.00011Te + 0.830$$
$$n = 48 \quad r = 0.664 \quad q^2 = 0.385 \quad s = 0.0533 \quad F = 36.21 \tag{1}$$

where n is the number of data points, r is the correlation coefficient, q^2 is the cross validated r^2 obtained by the leave-one-out (LOO) method, s is the standard error of the estimate, and F is the Fischer statistic.

Because the coefficient of Te in Eq. (1) is negative, antibacterial activity of synthesized compounds will decrease with increasing Te value. This is evidenced by the high antibacterial activity of compound **6** ($\mathrm{p}MIC_{\mathrm{ab}} = 1.45$ μM/cm^3) which has a low Te value ($Te = -5{,}411.83$).

To improve the value of the correlation coefficient (r), the electronic property total energy (Te) was coupled with the lipophilicity log P; as a result the r value increased from 0.664 to 0.706 (Eq. 2).

MLR-mt-QSAR model for antibacterial activity:

$$\mathrm{p}MIC_{\mathrm{ab}} = -0.020\ \log P - 0.000096Te + 0.791$$
$$n = 48 \quad r = 0.706 \quad q^2 = 0.203 \quad s = 0.0511 \quad F = 22.35. \tag{2}$$

The developed model was cross validated by the LOO method [36]. The q^2 value is <0.5 (Eq. 2), which showed that the developed model is invalid. According to the recommendations of Kim et al. [37], however, regression models are acceptable if the value of the standard deviation (SD, Table 1) is <0.3. Because the value of standard deviation is <0.3, the developed model (Eq. 2) is a valid one. Furthermore, the observed and predicted antibacterial activity values are close to each other (Table 12), so the mt-QSAR model for antibacterial activity (Eq. 2) is a valid one. The plot of predicted $\mathrm{p}MIC_{\mathrm{ab}}$ against observed $\mathrm{p}MIC_{\mathrm{ab}}$ (Fig. 1) also favours the developed model expressed by Eq. (2). Further, the plot of observed $\mathrm{p}MIC_{\mathrm{ab}}$ versus residual $\mathrm{p}MIC_{\mathrm{ab}}$ (Fig. 2) indicated that there was no systemic error in model development because the propagation of error was observed on both sides of zero [38].

Kier's second-order shape index (κ_2) was found to be the most dominating descriptor explaining the antifungal

Table 7 Anti-HIV potential of the synthesized compounds in MT-4 cells

Comp.	Avg. IC_{50}/μg cm^{-3}		Avg. CC_{50}/μg cm^{-3}
	HIV-1 (IIIB)	HIV-2 (ROD)	
1	>53	>53	53
2	>53	>53	53
3	>56	>56	56
4	>54	>54	54
5	>49	>49	49
6	>49	>49	49
7	>37	>37	37
8	>26	>2	26
9	>47	>4	47
10	>53	>53	53
11	>22	>22	22
12	>57	>57	57
13	>5	>5	5
14	>10	>10	10
15	>82	>8	82
16	>12	>12	12
17	>67	>67	67
18	>102	>102	≥102
19	>73	>102	≥73
20	>45	>45	≥45
21	>50	>50	50
22	>65	>65	65
23	>109	>104	≥109
24	>41	>41	41
25	>90	>90	≥90
26	>77	>77	77
27	>78	>78	78
28	>42	>42	≥42
29	>1	>1	1
30	>68	>68	68
31	>58	>58	58
32	>62	>62	62
33	>15	>15	15
34	>125	>125	>125
35	>10	>102	102
36	>12	>125	>125
37	>86	>86	86
38	>125	>125	>125
39	>4	>4	4
40	>17	>17	17
41	>59	>59	59
42	>64	>64	64
43	>73	>73	73
44	>74	>74	74
45	>70	>70	70
46	>69	>69	69
47	>59	>59	59
48	>113	>113	>113
Nevirapine	0.047	>4.00	>4.00
Azidothymidine, zidovudine, retrovir	0.001	0.0016	>25
Dideoxycytidine	0.29	0.30	>20
Dideoxyinosine, didanosine	2.9	4.6	>50

Table 8 Cytotoxicity (IC_{50}) of the synthesized compounds against human colon cell line HCT116 and breast cancer cell line MCF7

Comp.	IC_{50}/μg cm^{-3}		Comp.	IC_{50}/μg cm^{-3}	
	HCT 116	MCF 7		HCT 116	MCF 7
1	300	90	**25**	150	300
2	300	100	**26**	450	150
3	200	175	**27**	>1,000	210
4	350	120	**28**	60	NA
5	200	175	**29**	50	NA
6	300	200	**30**	NA	400
7	300	NA	**31**	100	NA
8	200	550	**32**	>1,000	NA
9	60	150	**33**	60	200
10	60	>1,000	**34**	>1,000	NA
11	70	175	**35**	>1,000	>1,000
12	70	300	**36**	NA	>1,000
13	40	NA	**37**	NA	NA
14	>1,000	>1,000	**38**	60	>1,000
15	110	35	**39**	NA	122
16	150	25	**40**	NA	>1,000
17	110	250	**41**	80	125
18	200	200	**42**	70	NA
19	190	200	**43**	700	112
20	19	18	**44**	300	110
21	270	200	**45**	80	122
22	30	120	**46**	>1,000	NA
23	60	200	**47**	350	NA
24	110	100	**48**	30	400
5-FU	6	0.67	**5-FU**	6	0.67

Data are mean values from three replicates

NA, not able to obtain IC_{50} after three independent tests

activity of the synthesized substituted hydrazide derivatives (Table 11).

LR-mt-QSAR model for antifungal activity:

Table 9 Values of selected descriptors used in QSAR studies of N′-[4-[(substituted imino)methyl]benzylidene]-substituted benzohydrazides

Comp.	log P	κ_1	κ_2	R	W	Te	LUMO	HOMO	μ
1	5.72	20.73	11.11	12.70	2,192.00	−4,220.71	−0.85	−8.66	4.92
2	6.24	21.70	11.25	13.11	2,412.00	−4,580.78	−0.87	−8.78	5.88
3	6.65	22.68	11.41	13.52	2,655.00	−4,532.36	−0.79	−8.65	4.62
4	6.65	22.68	11.41	13.51	2,676.00	−4,532.43	−0.80	−8.56	4.81
5	6.65	22.68	11.41	13.51	2,657.00	−4,532.43	−0.80	−8.62	5.18
6	6.19	24.64	12.30	14.42	3,262.00	−5,411.83	−1.54	−9.15	8.02
7	5.67	23.66	12.14	14.02	2,908.00	−5,051.73	−1.12	−9.01	4.25
8	5.67	23.66	12.14	14.01	2,968.00	−5,051.81	−1.23	−9.03	6.79
9	4.92	20.73	11.11	12.72	2,154.00	−4,181.28	−0.86	−8.69	5.62
10	5.43	21.70	11.25	13.13	2,372.00	−4,541.35	−0.88	−8.80	6.48
11	4.87	23.66	12.14	14.02	2,984.00	−5,012.41	−1.46	−9.15	8.50
12	5.85	22.68	11.41	13.52	2,615.00	−4,493.00	−0.79	−8.64	5.88
13	4.42	20.73	11.11	12.70	2,192.00	−4,081.72	−0.67	−8.59	6.19
14	4.94	21.70	11.25	13.10	2,432.00	−4,441.82	−0.79	−8.69	7.65
15	5.35	22.68	11.41	13.51	2,676.00	−4,393.43	−0.64	−8.47	5.74
16	5.35	22.68	11.41	13.51	2,657.00	−4,393.43	−0.64	−8.52	6.13
17	5.67	20.73	11.11	12.70	2,192.00	−4,016.52	−0.72	−8.65	6.30
18	6.19	21.70	11.25	13.11	2,412.00	−4,376.57	−0.79	−8.70	6.88
19	6.60	22.68	11.41	13.51	2,676.00	−4,328.23	−0.70	−8.51	5.78
20	6.19	21.70	11.25	13.10	2,412.00	−4,376.60	−0.84	−8.76	7.18
21	4.92	20.73	11.11	12.70	2,192.00	−4,181.26	−0.74	−8.66	5.05
22	5.43	21.70	11.25	13.11	2,412.00	−4,541.31	−0.80	−8.72	5.74
23	5.85	22.68	11.41	13.51	2,657.00	−4,492.96	−0.71	−8.58	5.03
24	5.67	23.66	12.14	14.02	2,914.00	−5,051.70	−1.46	−8.91	4.87
25	5.67	23.66	12.14	14.01	2,958.00	−5,051.75	−1.49	−8.89	4.32
26	5.15	22.68	12.00	13.61	2,718.00	−4,691.64	−1.64	−8.87	4.26
27	5.67	23.66	12.14	14.02	2,974.00	−5,051.70	−1.65	−8.93	4.61
28	4.37	23.66	12.14	14.01	3,028.00	−4,912.84	−1.37	−8.91	11.23
29	4.94	21.70	11.25	13.10	2,452.00	−4,441.83	−0.81	−8.65	7.45
30	4.37	23.66	12.14	14.01	2,968.00	−4,912.57	−1.21	−8.82	8.33
31	4.37	23.66	12.14	14.02	2,908.00	−4,912.49	−1.01	−8.83	8.99
32	4.84	24.64	12.30	14.42	3,205.00	−5,068.67	−1.12	−8.82	9.59
33	4.89	24.64	12.30	14.42	3,262.00	−5,272.84	−1.45	−8.92	11.12
34	5.43	21.70	11.25	13.10	2,432.00	−4,541.35	−0.85	−8.77	6.51
35	4.87	23.66	12.14	14.02	2,908.00	−5,012.26	−1.08	−8.93	4.77
36	4.87	23.66	12.14	14.01	3,028.00	−5,012.37	−1.41	−9.05	9.29
37	5.39	24.64	12.30	14.42	3,262.00	−5,372.37	−1.50	−9.07	9.38
38	4.87	23.66	12.14	14.01	2,968.00	−5,012.35	−1.19	−8.96	7.97
39	5.43	21.70	11.25	13.10	2,452.00	−4,541.37	−0.87	−8.72	6.04
40	5.43	21.70	11.25	13.11	2,412.00	−4,541.41	−0.94	−8.80	6.61
41	4.56	21.70	11.25	13.10	2,432.00	−4,553.12	−0.80	−8.71	8.13
45	5.08	22.68	11.41	13.51	2,695.00	−4,913.13	−0.92	−8.72	8.84
42	3.94	20.31	11.58	11.69	1,785.00	−3,882.10	−0.43	−8.66	5.44
43	4.51	21.70	11.87	13.20	2,492.00	−4,237.44	−0.46	−8.69	5.92
44	2.29	19.33	10.78	11.19	1,554.00	−3,891.18	−0.52	−8.73	6.48
46	2.98	17.36	9.21	10.19	1,160.00	−3,578.57	−0.51	−8.66	5.77
47	5.42	23.17	11.74	14.69	3,174.00	−4,621.09	−0.80	−8.31	6.22
48	3.55	19.33	10.78	11.19	1,554.00	−3,726.27	−0.43	−8.66	5.47

Table 10 Correlation matrix for the antibacterial activity of N′-[4-[(substituted imino)methyl]benzylidene]-substituted benzohydrazides

	$pMIC_{ab}$	log P	κ_2	R	W	Te	LUMO	HOMO	μ
$pMIC_{ab}$	1.000	0.453	0.572	0.636	0.651	−0.664	−0.546	−0.379	0.159
log P		1.000	0.258	0.530	0.468	−0.342	−0.207	0.046	−0.264
κ_2			1.000	0.874	0.891	−0.862	−0.746	−0.596	0.332
R				1.000	0.989	−0.892	−0.697	−0.390	0.319
W					1.000	−0.924	−0.743	−0.455	0.384
Te						1.000	0.862	0.703	−0.421
LUMO							1.000	0.791	−0.320
HOMO								1.000	−0.373
μ									1.000

Table 11 Correlation of different molecular descriptors with the antimicrobial activity of substituted hydrazide derivatives

	$pMIC_{ab}$	$pMIC_{af}$	$pMIC_{am}$
log P	0.453	0.322	0.473
MR	0.553	0.617	0.698
$^0\chi$	0.644	0.639	0.770
$^0\chi^v$	0.582	0.583	0.699
$^1\chi$	0.636	0.648	0.770
$^1\chi^v$	0.573	0.585	0.693
$^2\chi$	0.637	0.627	0.759
$^2\chi^v$	0.531	0.516	0.628
$^3\chi$	0.590	0.529	0.675
$^3\chi^v$	0.401	0.354	0.456
κ_1	0.641	0.645	0.771
κ_2	0.572	0.667	0.737
κ_3	0.407	0.562	0.572
$\kappa\alpha_1$	0.650	0.643	0.776
$\kappa\alpha_2$	0.566	0.652	0.725
$\kappa\alpha_3$	0.340	0.478	0.482
R	0.636	0.648	0.770
J	−0.409	−0.473	−0.525
W	0.651	0.647	0.778
Te	−0.664	−0.603	−0.763
Ele. E	−0.643	−0.612	−0.755
Nu. E	0.633	0.607	0.745
I.P.	0.379	0.317	0.421
LUMO	−0.546	−0.629	−0.700
HOMO	−0.379	−0.317	−0.421
μ	0.159	0.219	0.223

$$pMIC_{af} = 0.1007\kappa_2 + 0.332$$
$$n = 48 \quad r = 0.667 \quad q^2 = 0.389 \quad s = 0.065 \quad F = 36.84. \tag{3}$$

It is clearly evident from Eq. (3) that the antifungal activity of the synthesized compounds is positively correlated to Kier's second-order shape index (κ_2), i.e. the antifungal activity of the compounds will increase with increasing κ_2 value. High antifungal activity of compounds **25** and **27** ($pMIC_{af} = 1.66$) having a high κ_2 value ($\kappa_2 = 12.14$) illustrates the positive correlation. Very useful topological indices of the second generation are the κ indices of molecular shape and flexibility [39]. According to Kier, the shape of a molecule may be partitioned into attributes, each described by the count of bonds of various path lengths. The basis for devising a relative index of shape is given by the relationship between the number of paths of length l in the molecule i, ^lP_i, and 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 $^lP_{min}$. The first-order shape attribute, κ_1, is given by the expression:

$$\kappa_1 = n(n-1)^2/(^1P_i)^2.$$

The second and third-order kappa indices are defined as follows:

$$\kappa_2 = (n-1)(n-2)^2/(^2P_i)^2.$$

Coupling of the topological index Kier's second order shape index (κ_2) with the electronic property energy of the lowest unoccupied molecular orbital (LUMO) resulted in an increase in the r value from 0.667 to 0.695 (Eq. 4).

MLR-mt-QSAR model for antifungal activity:

$$pMIC_{af} = -0.067\kappa_2 - 0.0076\,\text{Lumo} + 0.641$$
$$n = 48 \quad r = 0.695 \quad q^2 = 0.191 \quad s = 0.063 \quad F = 21.07. \tag{4}$$

Although the q^2 value derived for Eq. (4) by the LOO method was <0.5, which made the model invalid, the low residual values observed for antifungal activity predictions using Eq. (4) (Table 12) and low standard deviation (Table 1) resulted in the QSAR model for antifungal activity being a valid one.

Table 12 Comparison of observed and predicted antimicrobial activity obtained by mt-QSAR models

Comp.	$pMIC_{ab}$/μM cm^{-3}			$pMIC_{af}$/μM cm^{-3}			$pMIC_{am}$/μM cm^{-3}		
	Obs.	Pre.	Res.	Obs.	Pre.	Res.	Obs.	Pre.	Res.
1	1.26	1.31	−0.05	1.46	1.45	0.01	1.34	1.36	−0.02
2	1.30	1.36	−0.06	1.50	1.46	0.04	1.38	1.38	0.00
3	1.39	1.36	0.03	1.49	1.47	0.02	1.43	1.39	0.04
4	1.29	1.36	−0.07	1.49	1.47	0.02	1.37	1.40	−0.03
5	1.39	1.36	0.03	1.49	1.47	0.02	1.43	1.39	0.04
6	1.45	1.44	0.01	1.55	1.58	−0.03	1.49	1.48	0.01
7	1.41	1.39	0.02	1.51	1.54	−0.03	1.45	1.43	0.02
8	1.31	1.39	−0.08	1.51	1.55	−0.04	1.39	1.44	−0.05
9	1.24	1.29	−0.05	1.44	1.45	−0.01	1.32	1.36	−0.04
10	1.28	1.34	−0.06	1.48	1.46	0.02	1.36	1.38	−0.02
11	1.29	1.37	−0.08	1.49	1.57	−0.08	1.37	1.46	−0.09
12	1.27	1.34	−0.07	1.47	1.47	0.00	1.35	1.39	−0.04
13	1.24	1.27	−0.03	1.44	1.44	0.00	1.32	1.35	−0.03
14	1.28	1.32	−0.04	1.48	1.46	0.02	1.36	1.38	−0.02
15	1.27	1.32	−0.05	1.47	1.46	0.01	1.35	1.39	−0.04
16	1.27	1.32	−0.05	1.47	1.45	0.02	1.35	1.39	−0.04
17	1.24	1.29	−0.05	1.44	1.44	0.00	1.32	1.35	−0.03
18	1.38	1.34	0.04	1.48	1.46	0.02	1.42	1.37	0.05
19	1.37	1.34	0.03	1.47	1.46	0.01	1.41	1.39	0.02
20	1.38	1.34	0.04	1.48	1.46	0.02	1.42	1.38	0.04
21	1.34	1.29	0.05	1.44	1.44	0.00	1.38	1.35	0.03
22	1.38	1.34	0.04	1.48	1.46	0.02	1.42	1.37	0.05
23	1.37	1.34	0.03	1.47	1.46	0.01	1.41	1.39	0.02
24	1.41	1.39	0.02	1.51	1.57	−0.06	1.45	1.45	0.00
25	1.41	1.39	0.02	1.66	1.57	0.09	1.51	1.45	0.06
26	1.37	1.35	0.02	1.62	1.57	0.05	1.47	1.44	0.03
27	1.41	1.39	0.02	1.66	1.58	0.08	1.51	1.46	0.05
28	1.39	1.35	0.04	1.64	1.56	0.08	1.49	1.45	0.04
29	1.28	1.32	−0.04	1.63	1.46	0.17	1.42	1.38	0.04
30	1.29	1.35	−0.06	1.64	1.55	0.09	1.43	1.44	−0.01
31	1.39	1.35	0.04	1.64	1.53	0.11	1.49	1.43	0.06
32	1.31	1.37	−0.06	1.51	1.55	−0.04	1.39	1.46	−0.07
33	1.43	1.40	0.03	1.53	1.58	−0.05	1.47	1.48	−0.01
34	1.38	1.34	0.04	1.48	1.46	0.02	1.42	1.38	0.04
35	1.39	1.37	0.02	1.34	1.54	−0.20	1.37	1.43	−0.06
36	1.39	1.37	0.02	1.49	1.56	−0.07	1.43	1.46	−0.03
37	1.43	1.42	0.01	1.53	1.58	−0.05	1.47	1.48	−0.01
38	1.39	1.37	0.02	1.49	1.55	−0.06	1.43	1.44	−0.01
39	1.28	1.34	−0.06	1.48	1.46	0.02	1.36	1.38	−0.02
40	1.38	1.34	0.04	1.48	1.47	0.01	1.42	1.38	0.04
41	1.36	1.32	0.04	1.31	1.46	−0.15	1.34	1.38	−0.04
45	1.40	1.36	0.04	1.50	1.48	0.02	1.44	1.40	0.04
42	1.31	1.24	0.07	1.41	1.45	−0.04	1.35	1.31	0.04
43	1.35	1.29	0.06	1.45	1.47	−0.02	1.39	1.36	0.03
44	1.09	1.21	−0.12	1.39	1.40	−0.01	1.21	1.29	−0.08
46	1.25	1.19	0.06	1.20	1.30	−0.10	1.23	1.26	−0.03
47	1.40	1.34	0.06	1.50	1.49	0.01	1.44	1.44	0.00
48	1.29	1.22	0.07	1.39	1.40	−0.01	1.33	1.29	0.04

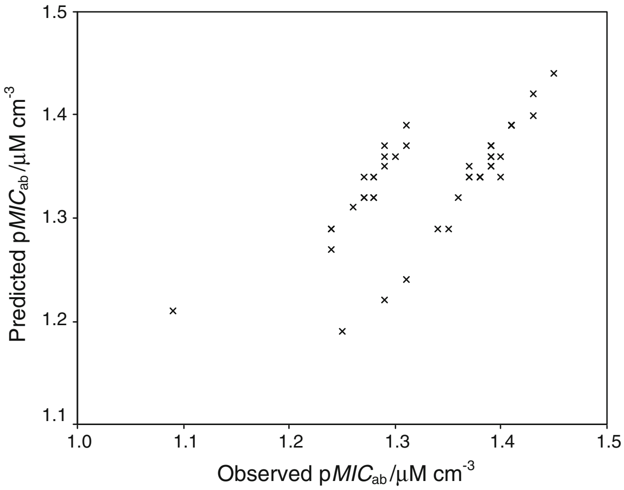


Fig. 1 Plot of predicted pMIC_{ab} values against observed pMIC_{ab} values for linear regression model expressed by Eq. (2)

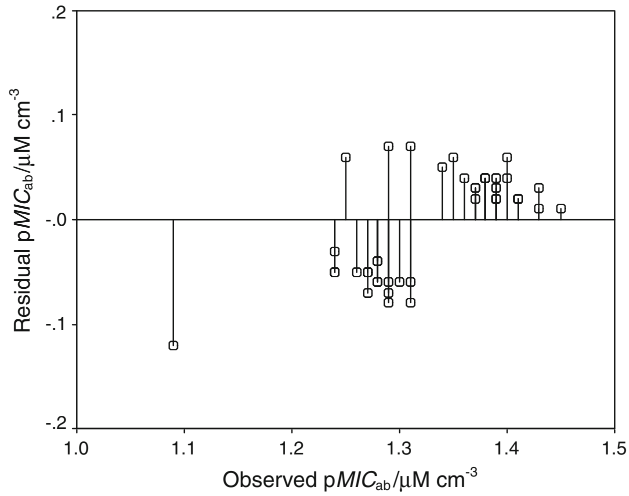


Fig. 2 Plot of residual pMIC_{ab} values against observed pMIC_{ab} values for linear regression model expressed by Eq. (2)

The antimicrobial activity of the synthesized hydrazide derivatives was best explained by a topological index, the Wiener index (W) (Table 11).

LR-mt-QSAR model for antimicrobial activity:

$$\text{p}MIC_{am} = -0.0001W + 1.114$$
$$n = 48 \quad r = 0.778 \quad q^2 = 0.567 \quad s = 0.040 \quad F = 70.62. \tag{5}$$

The Wiener index $W = W(G)$ of G is defined as the half sum of the elements of the distance matrix:

$$W = W(G) = 1/2\sum(D)ij \quad i = 1, \quad j = 1$$

where $(D)ij$ is the ijth element of the distance matrix which denotes the shortest graph-theoretical distance between sites i and j of G [31].

Similar to the antifungal activity, the antimicrobial activity of the synthesized compounds also positively correlated with the Wiener index (W) (Table 11). Addition of the electronic property energy of the lowest unoccupied molecular orbital (LUMO) to the topological index W resulted in the best model for describing antimicrobial activity of the synthesized compounds (Eq. 6).

MLR-mt-QSAR model for antimicrobial activity:

$$\text{p}MIC_{am} = -0.0008W + 0.052\text{LUMO} + 1.138$$
$$n = 48 \quad r = 0.799 \quad q^2 = 0.582 \quad s = 0.039 \quad F = 39.70. \tag{6}$$

The electronic property LUMO, which denotes the energy of the lowest unoccupied molecular orbital, is directly related to electron affinity and characterizes the sensitivity of the molecule to attack by a nucleophile. The contribution of LUMO to describing antimicrobial activity may be attributed to the interaction of hydrazide derivatives with a nucleophilic amino acid residue, for example cysteine, of microorganisms [40].

The validity and predictability of the QSAR model for antimicrobial activity, i.e. Eq. (6) is evidenced by the high q^2 value ($q^2 > 0.5$) obtained by the LOO method and the low residual activity values (Table 12). In summary, QSAR analysis indicated the importance of the topological index, the Wiener index (W), and the electronic property, the energy of the lowest unoccupied molecular orbital (LUMO), in describing the antimicrobial activity of N′-[4-[(substituted imino)methyl]benzylidene]-substituted benzohydrazides.

Generally, for QSAR studies, the biological activity of the compounds should span two to three orders of magnitude but in this study the range of antimicrobial activity of the synthesized compounds is within one order of magnitude. This is similar to results obtained by Bajaj et al. [41] who stated that the reliability of the QSAR model lies in its predictive ability even though the activity data are in a narrow range. When biological activity data is in a narrow range, a low standard deviation of the biological activity justifies its use in QSAR studies [42]. The low standard deviation observed in the antimicrobial activity data in Table 1 justifies its use in QSAR studies.

Conclusion

N′-[4-[(Substituted imino)methyl]benzylidene]-substituted benzohydrazides were synthesized and evaluated for their antimicrobial, antiviral, and anticancer potential. Antimicrobial activity results revealed that N′-[4-[(4-chlorophenylimino)methyl]benzylidene]-3-nitrobenzohydrazide (**25**) and N′-[4-[(2-chlorophenylimino)methyl]benzylidene]-4-nitro benzohydrazide (**27**) (pMIC_{am} = 1.51) were the most potent antimicrobial agents. In general, the synthesized compounds were bacteriostatic and fungistatic in action, because their MFC and MBC values were threefold higher than their MIC values. None of the synthesized compounds inhibited viral replication at sub-toxic concentrations. The results of anti-HIV screening against HIV-2 strain ROD indicated that compound **29** ($IC_{50} \geq 1$ µg/cm^3) was more potent than the standard drug nevirapine ($IC_{50} \geq 4$ µg/cm^3) and that **39** ($IC_{50} \geq 4$ µg/cm^3) was equipotent. Compound **20** was found to be the most effective anticancer agent against both HCT116 and MCF7 cancer cell lines, with IC_{50} values of 19 and 18 µg/cm^3, respectively. QSAR analysis indicated the importance of a topological index, the Wiener index (*W*), and an electronic property, the energy of the lowest unoccupied molecular orbital (LUMO), in describing the antimicrobial activity of the synthesized compounds.

Experimental

Starting materials were obtained from commercial sources and were used without further purification. Reaction progress was observed by thin-layer chromatography on commercial silica gel plates (Merck silica gel F_{254} on aluminum sheets) with chloroform–acetone 9:1 as mobile phase. Melting points were determined in open capillary tubes on a Sonar melting point apparatus. Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were determined using a Bruker Avance II 400 NMR spectrometer and are expressed in parts per million (δ, ppm) downfield from the internal standard, TMS. Compounds were dissolved in appropriate deuterated solvents. NMR data are provided with multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet) and number of protons. Infrared (IR) spectra were recorded on a Varian Resolutions Pro FTIR spectrometer. Elemental analysis was performed on a Perkin–Elmer 2400 C, H, N analyzer. Mass spectra were taken on a Waters Micromass Q-ToF Micro instrument.

General procedure for synthesis of N′-[4-[(substituted imino)methyl]benzylidene]-substituted benzohydrazides ***1–48***

A mixture of 80 mmol substituted benzoic acid and 34.04 g (43.14 cm^3, 0.74 mol) ethanol was heated under reflux in the presence of sulfuric acid until completion of the reaction. When the reaction was complete, the reaction mixture was added to 200 cm^3 ice-cold water and the ester formed was extracted with 50 cm^3 ether. The ether layer was separated and, on evaporation, yielded the crude ester which was then recrystallized from alcohol. Hydrazine hydrate (99 %, 0.015 mol) was added to an ethanolic solution of ester (0.01 mol) and the mixture was heated under reflux for 5 h. The reaction mixture was then cooled and the precipitate was isolated by filtration, washed with water, dried, and recrystallized from ethanol.

A solution of 6.70 g terephthaldehyde (50 mmol) in 50 cm^3 ethanol was added to a solution of the substituted benzoic acid hydrazide (synthesized above; 0.05 mol) in 50 cm^3 ethanol. The mixture was heated under reflux for 5 h. The reaction mixture was then left to cool at room temperature and the precipitated hydrazone was isolated by filtration, dried, and recrystallized from ethanol.

A solution of 50 mmol of the above synthesized hydrazone in 50 cm^3 DMF was added to a solution of 50 mmol substituted anilines/amines/naphthalen-1-amine in 50 cm^3 DMF. The mixture was heated under reflux for 5 h followed by cooling in an ice bath and the resulting product was isolated by filtration and purified.

N′-[4-[(Phenylimino)methyl]benzylidene]-4-chlorobenzoic acid hydrazide (**1**, $C_{21}H_{16}ClN_3O$)
Yield: 64.1 %; TLC: $R_f = 0.57$; IR (KBr): $\bar{\nu} = 1{,}649$ (C=O str., secondary amide), 1,506 (C=C skeletal str., phenyl nucleus), 820 (C–H out of plane bending, 1,4-disubstituted benzene ring), 730 (C–Cl str., ArCl), 706 (C–H out of plane bending, monosubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): δ = 8.32 (s, 1H, –CH=N), 7.92 (d, 2H, 4-chlorophenyl), 7.58 (d, 2H, benzylidene), 7.46 (m, 4H, 4-chlorophenyl, benzylidene), 7.29 (m, 5H, phenylimino) ppm; ^{13}C NMR (DMSO-d_6): δ = 163.3, 160.2, 153.5, 153.1, 143.1, 137.5, 136.0, 132.1, 130.4, 130.2, 129.5, 129.3, 128.5, 127.6, 127.6, 123.4, 122.5 ppm; MS (ES+, ToF): m/z = 363 ([M + 1]$^+$).

N′-[4-[(2-Chlorophenylimino)methyl]benzylidene]-4-chlorobenzoic acid hydrazide (**2**, $C_{21}H_{15}Cl_2N_3O$)
Yield: 61.2 %; TLC: $R_f = 0.75$; IR (KBr): $\bar{\nu} = 1{,}649$ (C=O str., secondary amide), 1,506 (C=C skeletal str., phenyl nucleus), 815 (C–H out of plane bending, 1,4-disubstituted benzene ring), 754 (C–H out of plane bending, 1,2-disubstituted benzene ring), 730 (C–Cl str., ArCl) cm^{-1}; ^{1}H NMR (MeOH-d_4): δ = 8.42 (s, 1H, –CH=N), 7.93 (m, 4H, 4-chlorophenyl, benzylidene), 7.93 (d, 2H, benzylidene), 7.59 (d, 2H, 4-chlorophenyl), 7.35 (m, 4H, 2-chlorophenyl) ppm; ^{13}C NMR (DMSO-d_6): δ = 163.0, 160.5, 143.4, 143.3, 137.4, 136.2, 132.4, 129.6, 129.1 ppm; MS (ES+, ToF): m/z = 397 ([M + 1]$^+$).

N′-[4-[(2,3-Dimethylphenylimino)methyl]benzylidene]-4-chlorobenzoic acid hydrazide (**3**, $C_{23}H_{20}ClN_3O$)
Yield: 65.5 %; TLC: $R_f = 0.79$; IR (KBr): $\bar{\nu} = 1{,}650$ (C=O str., secondary amide), 1,506 (C=C skeletal str., phenyl nucleus), 819 (C–H out of plane bending, 1,4-disubstituted benzene ring), 730 (C–C out of plane

bending, 1,2,3-trisubstituted benzene ring), 707 (C–Cl str., ArCl) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.12$ (s, 1H, –CH=N), 7.96 (m, 4H, 4-chlorophenyl, benzylidene), 7.83 (d, 2H, benzylidene), 7.44 (d, 2H, 4-chlorophenyl), 7.02 (m, 3H, 2,3-dimethylphenyl), 2.40 (s, 6H, $Ar(CH_3)_2$) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.6$, 160.5, 151.3, 143.0, 137.3, 136.4, 132.4, 129.2, 129.0, 128.8, 127.7, 119.0, 17.6, 9.2 ppm; MS (ES+, ToF): $m/z = 391$ ($[M + 1]^+$).

N'-[4-[(2,4-Dimethylphenylimino)methyl]benzylidene]-4-chlorobenzoic acid hydrazide (**4**, $C_{23}H_{20}ClN_3O$)
Yield: 68.3 %; TLC: $R_f = 0.52$; IR (KBr): $\bar{\nu} = 1{,}650$ (C=O str., secondary amide), 1,506 (C=C skeletal str., phenyl nucleus), 839 (C–H out of plane bending, 1,2,4-trisubstituted benzene ring), 818 (C–H out of plane bending, 1,4-disubstituted benzene ring), 730 (C–Cl str., ArCl) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.57$ (s, 1H, –CH=N), 7.94 (m, 4H, 4-chlorophenyl, benzylidene), 7.81 (d, 2H, benzylidene), 7.47 (d, 2H, 4-chlorophenyl), 7.01 (s, 1H, phenylimino), 7.00 (d, 2H, phenylimino), 2.36 (s, 6H, $Ar(CH_3)_2$) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.5$, 160.1, 148.5, 143.1, 137.2, 136.9, 136.3, 132.7, 132.4, 130.7, 129.4, 129.3, 127.1, 122.3 ppm; MS (ES+, ToF): $m/z = 391$ ($[M + 1]^+$).

N'-[4-[(2,5-Dimethylphenylimino)methyl]benzylidene]-4-chlorobenzoic acid hydrazide (**5**, $C_{23}H_{20}ClN_3O$)
Yield: 75.3 %; TLC: $R_f = 0.55$; IR (KBr): $\bar{\nu} = 1{,}649$ (C=O str., secondary amide), 1,506 (C=C skeletal str., phenyl nucleus), 815 (C–H out of plane bending, 1,4-disubstituted benzene ring), 730 (C–Cl str., ArCl) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.22$ (s, 1H, –CH=N), 7.93 (m, 4H, 4-chlorophenyl, benzylidene), 7.83 (d, 2H, benzylidene), 7.46 (d, 2H, 4-chlorophenyl), 7.01 (s, 1H, phenylimino), 7.00 (d, 2H, phenylimino), 2.69 (s, 6H, $Ar(CH_3)_2$) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.6$, 160.3, 151.0, 143.4, 137.3, 136.8, 136.0, 132.0, 130.2, 129.5, 129.1, 127.7, 123.9, 24.3, 15.6 ppm; MS (ES+, ToF): $m/z = 391$ ($[M + 1]^+$).

N'-[4-[(2-Chloro-4-nitrophenylimino)methyl]benzylidene]-4-chlorobenzoic acid hydrazide (**6**, $C_{21}H_{14}Cl_2N_4O_3$)
Yield: 72.0 %; TLC: $R_f = 0.61$; IR (KBr): $\bar{\nu} = 1{,}650$ (C=O str., secondary amide), 1,545 (NO_2 asym. str., Ar–NO_2), 1,506 (C=C skeletal str., phenyl nucleus), 839 (C–H out of plane bending, 1,2,4-trisubstituted benzene ring), 818 (C–H out of plane bending, 1,4-disubstituted benzene ring), 730 (C–Cl str., ArCl) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.65$ (s, 1H, –CH=N), 8.29 (s, 1H, $ArClNO_2$), 8.11 (d, 1H, $ArClNO_2$), 7.94 (m, 2H, benzylidene), 7.89 (m, 4H, 4-chlorophenyl, benzylidene), 7.59 (d, 1H, $ArClNO_2$), 7.45 (d, 2H, 4-chlorophenyl) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.4$, 160.1, 149.3, 148.4, 143.5, 137.4, 136.3, 132.3, 129.2, 129.0, 128.7, 125.0, 124.1, 120.6 ppm; MS (ES+, ToF): $m/z = 442$ ($[M + 1]^+$).

N'-[4-[(2-Nitrophenylimino)methyl]benzylidene]-4-chlorobenzoic acid hydrazide (**7**, $C_{21}H_{15}ClN_4O_3$)
Yield: 63.5 %; TLC: $R_f = 0.69$; IR (KBr): $\bar{\nu} = 1{,}649$ (C=O str., secondary amide), 1,548 (asym. str., NO_2), 1,514 (C=C skeletal str., phenyl nucleus), 814 (C–H out of plane bending, 1,4-disubstituted benzene ring), 752 (C–H out of plane bending, 1,2-disubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.72$ (s, 1H, –CH=N), 8.44 (d, 1H, $ArNO_2$), 8.04 (d, 2H, benzylidene), 7.99 (m, 4H, 4-chlorophenyl, benzylidene), 7.70 (m, 1H, $ArNO_2$), 7.59 (d, 2H, $ArNO_2$), 7.28 (d, 2H, 4-chlorophenyl) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 129.6$, 129.3, 128.3, 127.5, 119.8, 109.9, 107.3 ppm; MS (ES+, ToF): $m/z = 408$ ($[M + 1]^+$).

N'-[4-[(3-Nitrophenylimino)methyl]benzylidene]-4-chlorobenzoic acid hydrazide (**8**, $C_{21}H_{15}ClN_4O_3$)
Yield: 66.9 %; TLC: $R_f = 0.54$; IR (KBr): $\bar{\nu} = 1{,}649$ (C=N str., CH=N), 1,632 (C=O str., secondary amide), 1,520 (NO_2 asym. str., Ar-NO_2), 812 (C–H out of plane bending, 1,4-disubstituted benzene ring), 738 (C–Cl str., ArCl), 693 (C–C out of plane bending, 1,3-disubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.44$ (s, 1H, –CH=N), 8.17 (d, 2H, $ArNO_2$), 7.98 (d, 2H, benzylidene), 7.70 (m, 4H, 4-chlorophenyl, benzylidene), 7.60 (d, 2H, $ArNO_2$), 7.58 (d, 2H, 4-chlorophenyl), 7.53 (m, 1H, $ArNO_2$) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 191.6$, 149.5, 139.8, 129.7, 129.4, 128.4, 127.7, 120.1, 110.5, 107.8 ppm; MS (ES+, ToF): $m/z = 408$ ($[M + 1]^+$).

N'-[4-[(Phenylimino)methyl]benzylidene]-2-hydroxybenzoic acid hydrazide (**9**, $C_{21}H_{17}N_3O_2$)
Yield: 62.6 %; TLC: $R_f = 0.51$; IR (KBr): $\bar{\nu} = 1{,}650$ (C=N str., CH=N), 1,625 (C=O str., secondary amide), 1,585 (C=C skeletal str., phenyl nucleus), 1,359 (C–O str. and O–H in plane bending, phenol), 740 (C–H out of plane bending, 1,2-disubstituted benzene ring), 692 (C–C out of plane bending, monosubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 15.42$, 8.44 (s, 1H, –CH=N), 7.96 (d, 2H, benzylidene), 7.83 (d, 1H, 2-hydroxyphenyl), 7.82 (d, 2H, benzylidene), 7.40 (m, 1H, 2-hydroxyphenyl), 7.27 (m, 5H, phenylimino), 7.11 (m, 1H, 2-hydroxyphenyl), 6.94 (d, 1H, 2-hydroxyphenyl) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.2$, 160.4, 160.3, 159.1, 153.3, 143.2, 136.3, 133.5, 130.2, 129.8, 129.0, 127.3, 122.0, 121.7, 119.8 ppm; MS (ES+, ToF): $m/z = 344$ ($[M + 1]^+$).

N'-[4-[(2-Chlorophenylimino)methyl]benzylidene]-2-hydroxybenzoic acid hydrazide (**10**, $C_{21}H_{16}ClN_3O_2$)
Yield: 68.1 %; TLC: $R_f = 0.67$; IR (KBr): $\bar{\nu} = 1{,}650$ (C=N str., CH=N), 1,626 (C=O str., secondary amide), 1,585 (C=C skeletal str., phenyl nucleus), 1,359 (C–O str. and O–H in plane bending, phenol), 740 (C–H out of plane bending, 1,2-disubstituted benzene ring), 695 (C–Cl str., ArCl) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.41$ (s, 1H,

–CH=N), 7.81 (d, 2H, benzylidene), 7.81 (d, 1H, 2-hydroxyphenyl), 7.63 (d, 2H, benzylidene), 7.32 (m, 4H, phenylimino), 7.31 (m, 1H, 2-hydroxyphenyl), 7.10 (m, 1H, 2-hydroxyphenyl), 6.72 (d, 1H, 2-hydroxyphenyl) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 165.3$, 159.7, 147.9, 133.8, 129.6, 128.1, 127.6, 127.5, 118.6, 117.3, 114.8 ppm; MS (ES+, ToF): $m/z = 379$ ([M + 1]$^+$).

N′-[4-[(4-Nitrophenylimino)methyl]benzylidene]-2-hydroxybenzoic acid hydrazide (**11**, $C_{21}H_{16}N_4O_4$)
Yield: 69.3 %; TLC: $R_f = 0.56$; IR (KBr): $\bar{\nu} = 1{,}626$ (C=O str., secondary amide), 1,546 (asym. str., NO_2), 1,492 (C=C skeletal str., phenyl nucleus), 1,360 (C–O str. and O–H in plane bending, phenol), 829 (C–H out of plane bending, 1,4-disubstituted benzene ring), 740 (C–H out of plane bending, 1,2-disubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.43$ (s, 1H, –CH=N), 8.22 (d, 2H, phenylimino), 8.00 (d, 2H, benzylidene), 7.93 (d, 2H, benzylidene), 7.80 (d, 1H, 2-hydroxyphenyl), 7.51 (d, 2H, phenylimino), 7.44 (m, 1H, 2-hydroxyphenyl), 7.00 (m, 1H, 2-hydroxyphenyl), 6.97 (d, 1H, 2-hydroxyphenyl) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.0$, 160.7, 160.2, 159.3, 159.1, 146.9, 143.3, 136.5, 133.8, 129.9, 129.2, 123.5, 122.2, 121.5, 119.5 ppm; MS (ES+, ToF): $m/z = 389$ ([M + 1]$^+$).

N′-[4-[(2,5-Dimethylphenylimino)methyl]benzylidene]-2-hydroxybenzoic acid hydrazide (**12**, $C_{23}H_{21}N_3O_2$)
Yield: 62.2 %; TLC: $R_f = 0.63$; IR (KBr): $\bar{\nu} = 1{,}650$ (C=N str., CH=N), 1,626 (C=O str., secondary amide), 1,584 (C=C skeletal str., phenyl nucleus), 1,370 (C–O str. and O–H in plane bending, phenol), 740 (C–H out of plane bending, 1,2-disubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 9.84$ (s, 1H, phenylimino), 8.28 (s, 1H, –CH=N), 7.93 (d, 2H, benzylidene), 7.85 (d, 2H, benzylidene), 7.81 (d, 1H, 2-hydroxyphenyl), 7.46 (m, 1H, 2-hydroxyphenyl), 7.01 (m, 1H, 2-hydroxyphenyl), 7.00 (d, 2H, phenylimino), 6.94 (d, 1H, 2-hydroxyphenyl), 2.68 (s, 6H, $Ar(CH_3)_2$) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.1$, 160.4, 159.2, 151.2, 136.7, 133.6, 130.1, 129.9, 127.8, 123.5, 121.7, 119.6, 24.2, 15.8 ppm; MS (ES+, ToF): $m/z = 372$ ([M + 1]$^+$).

N′-[4-[(Phenylimino)methyl]benzylidene]-4-aminobenzoic acid hydrazide (**13**, $C_{21}H_{18}N_4O$)
Yield: 61.0 %; TLC: $R_f = 0.72$; IR (KBr): $\bar{\nu} = 1{,}707$ (C=N str., CH=N), 1,616 (C=O str., secondary amide), 1,584 (C=C skeletal str., phenyl nucleus), 1,310 (C–N str., Ar–NH_2), 829 (C–H out of plane bending, 1,4-disubstituted benzene ring), 693 (C–H out of plane bending, monosubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.67$ (s, 1H, –CH=N), 8.10 (d, 2H, benzylidene), 7.92 (d, 2H, benzylidene), 7.59 (d, 2H, 4-aminophenyl), 7.33 (m, 5H, phenylimino), 6.99 (d, 2H, 4-aminophenyl) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.3$, 160.0, 153.3, 151.6, 143.1, 136.1, 130.1, 129.2, 128.3, 127.0, 124.1, 122.4, 116.0 ppm; MS (ES+, ToF): $m/z = 343$ ([M + 1]$^+$).

N′-[4-[(3-Chlorophenylimino)methyl]benzylidene]-4-aminobenzoic acid hydrazide (**14**, $C_{21}H_{17}ClN_4O$)
Yield: 73.8 %; TLC: $R_f = 0.55$; IR (KBr): $\bar{\nu} = 1{,}675$ (C=N str., CH=N), 1,624 (C=O str., secondary amide), 1,584 (C=C skeletal str., phenyl nucleus), 1,303 (C–N str., Ar-NH_2), 837 (C–H out of plane bending, 1,4-disubstituted benzene ring), 730 (C–Cl str., ArCl), 682 (C–C out of plane bending, 1,3-disubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.68$ (s, 1H, –CH=N), 7.93 (d, 2H, benzylidene), 7.80 (d, 2H, benzylidene), 7.74 (d, 2H, 4-aminophenyl), 7.34 (s, 1H, phenylimino), 7.26 (d, 2H, phenylimino), 7.02 (m, 1H, phenylimino), 6.64 (d, 2H, 4-aminophenyl) ppm; ^{13}C NMR (DMSO-d_6): $\delta =$ 163.1, 160.3, 154.6, 151.8, 143.1, 136.0, 135.9, 131.7, 129.1, 128.4, 127.3, 124.2, 122.7, 120.2, 116.2 ppm; MS (ES+, ToF): $m/z = 378$ ([M + 1]$^+$).

N′-[4-[(2,4-Dimethylphenylimino)methyl]benzylidene]-4-aminobenzoic acid hydrazide (**15**, $C_{23}H_{22}N_4O$)
Yield: 49.7 %; TLC: $R_f = 0.67$; IR (KBr): $\bar{\nu} = 1{,}706$ (C=N str., CH=N), 1,623 (C=O str., secondary amide), 1,592 (C=C skeletal str., phenyl nucleus), 1,308 (C–N str., Ar-NH_2), 884 (C–H out of plane bending, 1,2,4-trisubstituted benzene ring), 802 (C–H out of plane bending, 1,4-disubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.57$ (s, 1H, –CH=N), 7.93 (d, 2H, benzylidene), 7.72 (d, 2H, 4-aminophenyl), 7.72 (d, 2H, benzylidene), 7.10 (d, 2H, phenylimino), 6.94 (s, 1H, phenylimino), 6.68 (d, 2H, 4-aminophenyl), 2.37 (s, 6H, $Ar(CH_3)_2$) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.3$, 160.5, 160.2, 151.6, 148.2, 143.2, 136.6, 136.2, 132.4, 130.7, 129.4, 128.2, 127.0, 124.0, 116.3, 112.2, 24.8 ppm; MS (ES+, ToF): $m/z = 371$ ([M + 1]$^+$).

N′-[4-[(2,5-Dimethylphenylimino)methyl]benzylidene]-4-aminobenzoic acid hydrazide (**16**, $C_{23}H_{22}N_4O$)
Yield: 66.2 %; TLC: $R_f = 0.69$; IR (KBr): $\bar{\nu} = 1{,}677$ (C=N str., CH=N), 1,618 (C=O str., secondary amide), 1,571 (C=C skeletal str., phenyl nucleus), 1,292 (C–N str., Ar-NH_2), 804 (C–H out of plane bending, 1,4-disubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.52$ (s, 1H, –CH=N), 7.93 (d, 2H, benzylidene), 7.82 (d, 2H, benzylidene), 7.72 (d, 2H, 4-aminophenyl), 6.99 (d, 2H, phenylimino), 6.94 (s, 1H, phenylimino), 6.86 (d, 2H, 4-aminophenyl), 2.37 (s, 6H, $Ar(CH_3)_2$) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 158.8$, 149.9, 138.5, 135.8, 129.3, 128.8, 128.4, 126.5, 118.2 ppm; MS (ES+, ToF): $m/z =$ 371 ([M + 1]$^+$).

N'-[4-[(Phenylimino)methyl]benzylidene]-4-methylbenzoic acid hydrazide (**17**, $C_{22}H_{19}N_3O$)
Yield: 64.0 %; TLC: $R_f = 0.38$; IR (KBr): $\bar{\nu} = 1{,}690$ (C=N str., CH=N), 1,617 (C=O str., secondary amide), 1,507 (C=C skeletal str., phenyl nucleus), 827 (C–H out of plane bending, 1,4-disubstituted benzene ring) cm^{-1}; ^1H NMR (MeOH-d_4): $\delta = 8.42$ (s, 1H, –N=CH), 7.98 (d, 2H, benzylidene), 7.86 (d, 2H, 4-methylbenzohydrazide), 7.83 (d, 2H, benzylidene), 7.65 (m, 5H, phenylimino), 7.22 (d, 2H, 4-methylbenzohydrazide) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.2$, 160.2, 153.5, 143.1, 141.4, 136.3, 130.3, 129.5, 129.4, 127.6, 127.5, 122.4, 24.6 ppm; MS (ES+, ToF): $m/z = 342$ ($[M + 1]^+$).

N'-[4-[(2-Chlorophenylimino)methyl]benzylidene]-4-methylbenzoic acid hydrazide (**18**, $C_{22}H_{18}ClN_3O$)
Yield: 68.4 %; TLC: $R_f = 0.73$; IR (KBr): $\bar{\nu} = 1{,}646$ (C=N str., CH=N), 1,611 (C=O str., secondary amide), 1,502 (C=C skeletal str., phenyl nucleus), 830 (C–H out of plane bending, 1,4-disubstituted benzene ring), 745 (C–H out of plane bending, 1,2-disubstituted benzene ring), 704 (C–Cl str., ArCl) cm^{-1}; ^1H NMR (MeOH-d_4): $\delta = 7.96$ (s, 1H, –CH=N), 7.94 (d, 2H, benzylidene), 7.90 (d, 2H, benzylidene), 7.88 (d, 2H, 4-methylbenzohydrazide), 7.40 (d, 2H, 4-methylbenzohydrazide), 7.40 (d, 3H, phenylimino), 7.22 (m, 1H, phenylimino), 2.46 (s, 3H, $ArCH_3$) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.0$, 160.2, 143.4, 143.4, 141.1, 136.5, 130.5, 129.7, 129.3, 128.7, 127.5, 127.4, 124.7, 12.5 ppm; MS (ES+, ToF): $m/z = 377$ ($[M + 1]^+$).

N'-[4-[(2,4-Dimethylphenylimino)methyl]benzylidene]-4-methylbenzoic acid hydrazide (**19**, $C_{24}H_{23}N_3O$)
Yield: 61.7 %; TLC: $R_f = 0.50$; IR (KBr): $\bar{\nu} = 1{,}652$ (C=N str., CH=N), 1,630 (C=O str., secondary amide), 1,505 (C=C skeletal str., phenyl nucleus), 917 (C–H out of plane bending, 1,2,4-trisubstituted benzene ring), 832 (C–H out of plane bending, 1,4-disubstituted benzene ring) cm^{-1}; ^1H NMR (MeOH-d_4): $\delta = 8.00$ (s, 1H, –N=CH), 7.99 (d, 2H, benzylidene), 7.87 (d, 2H, 4-methylbenzohydrazide), 7.86 (d, 2H, benzylidene), 7.39 (d, 2H, 4-methylbenzohydrazide), 6.84 (d, 2H, phenylimino), 6.08 (s, 1H, phenylimino), 2.36 (s, 6H, $-Ar(CH_3)_2$) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.3$, 160.5, 160.4, 148.3, 143.6, 141.0, 136.8, 136.7, 132.3, 130.7, 129.5, 129.4, 127.4, 127.2, 122.2, 24.9 ppm; MS (ES+, ToF): $m/z = 370$ ($[M + 1]^+$).

N'-[4-[(3-Chlorophenylimino)methyl]benzylidene]-3-methylbenzoic acid hydrazide (**20**, $C_{22}H_{18}ClN_3O$)
Yield: 62.3 %; TLC: $R_f = 0.82$; IR (KBr): $\bar{\nu} = 1{,}656$ (C=N str., CH=N), 1,619 (C=O str., secondary amide), 1,506 (C=C skeletal str., phenyl nucleus), 732 (C–Cl str., ArCl), 682 (C–C out of plane bending, 1,3-disubstituted benzene ring) cm^{-1}; ^1H NMR (MeOH-d_4): $\delta = 8.00$ (s, 1H, –CH=N), 7.96 (d, 1H, 3-methylbenzohydrazide), 7.96 (d, 2H, benzylidene), 7.81 (d, 2H, benzylidene), 7.78 (s, 1H, 3-methylbenzohydrazide), 7.46 (d, 1H, 3-methylbenzohydrazide), 7.33 (s, 1H, phenylimino), 7.29 (m, 1H, phenylimino), 7.21 (d, 2H, phenylimino), 7.03 (m, 1H, 3-methylbenzohydrazide), 2.47 (s, 3H, $ArCH_3$) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.4$, 160.7, 154.6, 143.1, 138.3, 136.6, 135.5, 134.2, 132.7, 131.2, 129.8, 128.5, 127.5, 127.4, 124.6, 122.8, 120.3, 24.7 ppm; MS (ES+, ToF): $m/z = 377$ ($[M + 1]^+$).

N'-[4-[(Phenylimino)methyl]benzylidene]-4-hydroxybenzoic acid hydrazide (**21**, $C_{21}H_{17}N_3O_2$)
Yield: 65.7 %; TLC: $R_f = 0.64$; IR (KBr): $\bar{\nu} = 1{,}697$ (C=N str., CH=N), 1,618 (C=O str., secondary amide), 1,584 (C=C skeletal str., phenyl nucleus), 1,388 (C–O str. and O–H in plane bending, phenol), 827 (C–H out of plane bending, 1,4-disubstituted benzene ring), 691 (C–C out of plane bending, monosubstituted benzene ring) cm^{-1}; ^1H NMR (MeOH-d_4): $\delta = 8.09$ (s, 1H, –CH=N), 7.95 (d, 2H, benzylidene), 7.85 (d, 2H, benzylidene), 7.79 (d, 2H, 4-hydroxybenzohydrazide), 7.32 (m, 5H, phenylimino), 6.85 (d, 2H, 4-hydroxybenzohydrazide) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.2$, 161.6, 160.3, 153.2, 143.3, 136.2, 130.3, 129.4, 128.7, 128.5, 127.5, 122.4, 116.2 ppm; MS (ES+, ToF): $m/z = 344$ ($[M + 1]^+$).

N'-[4-[(2-Chlorophenylimino)methyl]benzylidene]-4-hydroxybenzoic acid hydrazide (**22**, $C_{21}H_{16}ClN_3O_2$)
Yield: 78.2 %; TLC: $R_f = 0.62$; IR (KBr): $\bar{\nu} = 1{,}687$ (C=N str., CH=N), 1,620 (C=O str., secondary amide), 1,576 (C=C skeletal str., phenyl nucleus), 1,392 (C–O str. and O–H in plane bending, phenol), 817 (C–H out of plane bending, 1,4-disubstituted benzene ring), 772 (C–H out of plane bending, 1,2-disubstituted benzene ring), 718 (C–Cl str., ArCl) cm^{-1}; ^1H NMR (MeOH-d_4): $\delta = 8.37$ (s, 1H, –CH=N), 7.93 (d, 2H, benzylidene), 7.81 (d, 2H, benzylidene), 7.76 (d, 2H, 4-hydroxybenzohydrazide), 7.33 (m, 3H, phenylimino), 7.22 (m, 1H, phenylimino), 6.84 (d, 2H, 4-hydroxybenzohydrazide) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.0$, 161.3, 160.4, 151.4, 143.4, 143.1, 136.9, 136.3, 130.3, 130.2, 129.5, 128.6, 128.6, 128.6, 127.8, 127.5, 123.9, 123.3, 116.0, 24.2, 15.8 ppm; MS (ES+, ToF): $m/z = 379$ ($[M + 1]^+$).

N'-[4-[(2,5-Dimethylphenylimino)methyl]benzylidene]-4-hydroxybenzoic acid hydrazide (**23**, $C_{23}H_{21}N_3O_2$)
Yield: 81.5 %; TLC: $R_f = 0.57$; IR (KBr): $\bar{\nu} = 1{,}688$ (C=N str., CH=N), 1,620 (C=O str., secondary amide), 1,576 (C=C skeletal str., phenyl nucleus), 1,391 (C–O str. and O–H in plane bending, phenol), 824 (C–H out of plane bending, 1,4-disubstituted benzene ring) cm^{-1}; ^1H NMR (MeOH-d_4): $\delta = 8.01$ (s, 1H, –CH=N), 7.91 (d, 2H, benzylidene), 7.85 (d, 2H, benzylidene), 7.76 (d, 2H, 4-hydroxybenzohydrazide), 7.48 (d, 2H, 4-hydroxybenzohydrazide), 7.32 (d, 2H, phenylimino), 7.02 (s, 1H, phenylimino), 2.36 (s, 6H, $-Ar(CH_3)_2$) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.2$, 161.5, 160.1, 143.5, 136.1, 129.7,

128.7, 128.4, 116.2 ppm; MS (ES+, ToF): $m/z = 372$ ($[M + 1]^+$).

N′-[4-[(2-Chlorophenylimino)methyl]benzylidene]-3-nitrobenzoic acid hydrazide (**24**, $C_{21}H_{15}ClN_4O_3$)
Yield: 84.0 %; TLC: $R_f = 0.81$; IR (KBr): $\bar{\nu} = 1,650$ (C=N str., CH=N), 1,614 (C=O str., secondary amide), 1,550 (asym. str., NO_2), 1,504 (C=C skeletal str., phenyl nucleus), 768 (C–H out of plane bending, 1,2-disubstituted benzene ring), 753 (C–Cl str., ArCl), 721 (C–C out of plane bending, 1,3-disubstituted benzene ring) cm^{-1}; ^1H NMR (MeOH-*d₄*): $\delta = 8.92$ (s, 1H, 3-nitrobenzohydrazide), 8.61 (d, 1H, 3-nitrobenzohydrazide), 8.54 (d, 1H, 3-nitrobenzohydrazide), 8.44 (s, 1H, –CH=N), 7.95 (d, 2H, benzylidene), 7.89 (m, 1H, 3-nitrobenzohydrazide), 7.63 (d, 2H, benzylidene), 7.36 (m, 3H, phenylimino), 7.21 (m, 1H, phenylimino) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.2$, 160.2, 148.6, 143.3, 143.1, 136.3, 135.2, 133.9, 130.4, 129.9, 129.4, 128.7, 127.5, 124.1, 123.3, 122.5 ppm; MS (ES+, ToF): $m/z = 408$ ($[M + 1]^+$).

N′-[4-[(4-Chlorophenylimino)methyl]benzylidene]-3-nitrobenzoic acid hydrazide (**25**, $C_{21}H_{15}ClN_4O_3$)
Yield: 76.7 %; TLC: $R_f = 0.54$; IR (KBr): $\bar{\nu} = 1,651$ (C=N str., CH=N), 1,614 (C=O str., secondary amide), 1,552 (asym. str., NO_2), 1,506 (C=C skeletal str., phenyl nucleus), 815 (C–H out of plane bending, 1,4-disubstituted benzene ring), 721 (C–Cl str., ArCl) cm^{-1}; ^1H NMR (MeOH-d_4): $\delta = 8.90$ (s, 1H, 3-nitrobenzohydrazide), 8.43 (d, 1H, 3-nitrobenzohydrazide), 8.42 (s, 1H, –CH=N), 8.07 (d, 1H, 3-nitrobenzohydrazide), 7.97 (d, 2H, benzylidene), 7.82 (m, 1H, 3-nitrobenzohydrazide), 7.61 (d, 2H, benzylidene), 7.31 (d, 2H, phenylimino), 7.19 (d, 2H, phenylimino) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.3$, 160.0, 151.0, 148.9, 143.1, 136.0, 135.3, 133.8, 132.6, 130.1, 129.7, 129.5, 124.2, 123.8, 122.6 ppm; MS (ES+, ToF): $m/z = 408$ ($[M + 1]^+$).

N′-[4-[(Phenylimino)methyl]benzylidene]-4-nitrobenzoic acid hydrazide (**26**, $C_{21}H_{16}N_4O_3$)
Yield: 69.3 %; TLC: $R_f = 0.70$; IR (KBr): $\bar{\nu} = 1,657$ (C=N str., CH=N), 1,561 (asym. str., NO_2), 1,516 (C=C skeletal str., phenyl nucleus), 820 (C–H out of plane bending, 1,4-disubstituted benzene ring), 702 (C–C out of plane bending, monosubstituted benzene ring) cm^{-1}; ^1H NMR (MeOH-d_4): $\delta = 8.42$ (d, 2H, 4-nitrobenzohydrazide), 8.42 (s, 1H, –CH=N), 8.19 (d, 2H, 4-nitrobenzohydrazide), 8.01 (d, 2H, benzylidene), 7.89 (d, 2H, benzylidene), 7.19 (m, 5H, phenylimino) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 161.9$, 149.8, 141.8, 129.6, 129.0, 128.8, 127.5, 123.3, 122.6, 121.2 ppm; MS (ES+, ToF): $m/z = 373$ ($[M + 1]^+$).

N′-[4-[(2-Chlorophenylimino)methyl]benzylidene]-4-nitrobenzoic acid hydrazide (**27**, $C_{21}H_{15}ClN_4O_3$)
Yield: 67.4 %; TLC: $R_f = 0.78$; IR (KBr): $\bar{\nu} = 1,657$ (C=N str., CH=N), 1,562 (asym. str., NO_2), 1,515 (C=C skeletal str., phenyl nucleus), 821 (C–H out of plane bending, 1,4-disubstituted benzene ring), 702 (C–Cl str., ArCl) cm^{-1}; ^1H NMR (MeOH-d_4): $\delta = 8.57$ (s, 1H, –N=CH), 8.32 (d, 2H, 4-nitrobenzohydrazide), 8.01 (d, 2H, 4-nitrobenzohydrazide), 8.00 (d, 2H, benzylidene), 7.76 (d, 2H, benzylidene), 7.38 (m, 3H, phenylimino), 7.22 (m, 1H, phenylimino) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.3$, 162.1, 148.9, 143.1, 135.3, 133.8, 129.8, 129.7, 129.2, 124.2, 123.4, 122.6 ppm; MS (ES+, ToF): $m/z = 408$ ($[M + 1]^+$).

N′-[4-[(4-Nitrophenylimino)methyl]benzylidene]-4-aminobenzoic acid hydrazide (**28**, $C_{21}H_{17}N_5O_3$)
Yield: 83.2 %; TLC: $R_f = 0.42$; IR (KBr): $\bar{\nu} = 1,689$ (C=N str., CH=N), 1,619 (C=O str., secondary amide), 1,505 (asym. str., NO_2), 1,275 (C–N str., Ar–NH_2), 823 (C–H out of plane bending, 1,4-disubstituted benzene ring) cm^{-1}; ^1H NMR (MeOH-d_4): $\delta = 8.33$ (s, 1H, –N=CH), 8.12 (d, 2H, phenylimino), 7.89 (d, 2H, benzylidene), 7.84 (d, 2H, benzylidene), 7.70 (d, 2H, 4-aminophenyl), 7.60 (d, 2H, phenylimino), 6.66 (d, 2H, 4-aminophenyl) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.4$, 160.1, 159.5, 151.8, 146.8, 143.2, 136.0, 129.3, 128.4, 124.0, 123.4, 123.3, 122.7, 122.5, 116.1 ppm; MS (ES+, ToF): $m/z = 388$ ($[M + 1]^+$).

N′-[4-[(4-Chlorophenylimino)methyl]benzylidene]-4-aminobenzoic acid hydrazide (**29**, $C_{21}H_{17}ClN_4O$)
Yield: 66.7 %; TLC: $R_f = 0.44$; IR (KBr): $\bar{\nu} = 1,700$ (C=N str., CH=N), 1,618 (C=O str., secondary amide), 1,274 (C–N str., Ar–NH_2), 828 (C–H out of plane bending, 1,4-disubstituted benzene ring), 729 (C–Cl str., ArCl) cm^{-1}; ^1H NMR (MeOH-d_4): $\delta = 8.33$ (s, 1H, –N=CH), 8.12 (d, 2H, phenylimino), 7.98 (d, 2H, benzylidene), 7.83 (d, 2H, benzylidene), 7.74 (d, 2H, 4-aminophenyl), 7.60 (d, 2H, phenylimino), 6.64 (d, 2H, 4-aminophenyl), 4.31 (s, 2H, –ArNH_2) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.7$, 160.4, 151.5, 151.5, 143.5, 136.0, 132.6, 130.4, 130.3, 129.1, 128.6, 124.3, 123.9, 123.5, 116.4 ppm; MS (ES+, ToF): $m/z = 378$ ($[M + 1]^+$).

N′-[4-[(3-Nitrophenylimino)methyl]benzylidene]-4-aminobenzoic acid hydrazide (**30**, $C_{21}H_{17}N_5O_3$)
Yield: 67.4 %; TLC: $R_f = 0.53$; IR (KBr): $\bar{\nu} = 1,698$ (C=N str., CH=N), 1,619 (C=O str., secondary amide), 1,524 (asym. str., NO_2), 1,274 (C–N str., Ar–NH_2), 826 (C–H out of plane bending, 1,4-disubstituted benzene ring), 697 (C–C out of plane bending, 1,3-disubstituted benzene ring) cm^{-1}; ^1H NMR (MeOH-d_4): $\delta = 8.42$ (s, 1H, –N=CH), 8.31 (s, 1H, phenylimino), 8.11 (d, 1H, phenylimino), 7.99 (d, 2H, benzylidene), 7.85 (d, 2H, benzylidene), 7.79 (d, 1H, phenylimino), 7.78 (d, 2H, 4-aminophenyl), 7.50 (m, 1H, phenylimino), 6.67 (d, 2H, 4-aminophenyl), 4.29 (s, 2H, –NH_2 of ArNH_2) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.1$, 160.3, 154.3, 151.6, 149.6, 143.3, 136.1, 131.2, 129.4, 128.7, 128.0, 124.2, 119.9, 117.3, 116.3 ppm; MS (ES+, ToF): $m/z = 388$ ($[M + 1]^+$).

N'-[4-[(2-Nitrophenylimino)methyl]benzylidene]-4-aminobenzoic acid hydrazide (**31**, $C_{21}H_{17}N_5O_3$)
Yield: 60.5 %; TLC: $R_f = 0.56$; IR (KBr): $\bar{\nu} = 1{,}697$ (C=N str., CH=N), 1,619 (C=O str., secondary amide), 1,504 (asym. str., NO_2), 1,275 (C–N str., Ar–NH_2), 826 (C–H out of plane bending, 1,4-disubstituted benzene ring), 744 (C–H out of plane bending, 1,2-disubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.52$ (d, 2H, phenylimino), 8.26 (s, 1H, –N=CH), 8.11 (d, 1H, phenylimino), 7.98 (d, 2H, benzylidene), 7.85 (d, 2H, benzylidene), 7.79 (m, 1H, phenylimino), 7.77 (d, 2H, 4-aminophenyl), 7.38 (m, 1H, phenylimino), 6.66 (d, 2H, 4-aminophenyl), 4.32 (s, 2H, –NH_2 of ArNH_2) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.0$, 160.0, 151.8, 148.5, 143.5, 141.8, 136.4, 136.3, 129.6, 128.3, 128.2, 124.0, 123.3, 122.6, 116.5 ppm; MS (ES+, ToF): $m/z = 388$ ($[M + 1]^+$).

N'-[4-[(2-Methyl-5-nitrophenylimino)methyl]benzylidene]-4-aminobenzoic acid hydrazide (**32**, $C_{22}H_{19}N_5O_3$)
Yield: 40.6 %; TLC: $R_f = 0.54$; IR (KBr): $\bar{\nu} = 1{,}701$ (C=N str., CH=N), 1,623 (C=O str., secondary amide), 1,509 (asym. str., NO_2), 1,270 (C–N str., Ar–NH_2), 829 (C–H out of plane bending, 1,4-disubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.46$ (s, 1H, –N=CHAr), 8.32 (d, 1H, phenylimino), 8.21 (d, 2H, phenylimino), 7.93 (d, 2H, benzylidene), 7.83 (d, 2H, benzylidene), 7.72 (d, 2H, 4-aminophenyl), 6.64 (d, 2H, 4-aminophenyl), 2.07 (s, 3H, –ArCH_3) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.1$, 160.4, 152.4, 151.3, 146.9, 143.6, 136.7, 136.0, 131.6, 129.5, 128.0, 124.1, 119.4, 117.2, 116.3, 15.5 ppm; MS (ES+, ToF): $m/z = 402$ ($[M + 1]^+$).

N'-[4-[(2-Chloro-4-nitrophenylimino)methyl]benzylidene]-4-aminobenzoic acid hydrazide (**33**, $C_{21}H_{16}ClN_5O_3$)
Yield: 63.7 %; TLC: $R_f = 0.36$; IR (KBr): $\bar{\nu} = 1{,}703$ (C=N str., CH=N), 1,620 (C=O str., secondary amide), 1,505 (asym. str., NO_2), 1,272 (C–N str., Ar–NH_2), 885 (C–H out of plane bending, 1,2,4-trisubstituted benzene ring), 828 (C–H out of plane bending, 1,4-disubstituted benzene ring), 723 (C–Cl str., Ar–Cl) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.65$ (s, 1H, –CH=N), 8.22 (s, 1H, phenylimino), 8.11 (d, 1H, phenylimino), 8.05 (d, 2H, benzylidene), 7.84 (d, 2H, benzylidene), 7.76 (d, 2H, 4-aminophenyl), 7.55 (d, 1H, phenylimino), 6.65 (d, 2H, 4-aminophenyl), 4.31 (s, 2H, –ArNH_2) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.5$, 160.5, 151.1, 149.5, 148.6, 143.8, 136.1, 129.3, 128.9, 128.2, 125.4, 124.7, 124.5, 120.4, 116.4 ppm; MS (ES+, ToF): $m/z = 423$ ($[M + 1]^+$).

N'-[4-[(3-Chlorophenylimino)methyl]benzylidene]-4-hydroxybenzoic acid hydrazide (**34**, $C_{21}H_{16}ClN_3O_2$)
Yield: 60.9 %; TLC: $R_f = 0.57$; IR (KBr): $\bar{\nu} = 1{,}699$ (C=N str., CH=N), 1,618 (C=O str., secondary amide), 1,581 (C=C skeletal str., phenyl nucleus), 1,390 (C–O str. and O–H in plane bending, phenol), 826 (C–H out of plane bending, 1,4-disubstituted benzene ring), 710 (C–Cl str., Ar–Cl), 683 (C–C out of plane bending, 1,3-disubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.65$ (s, 1H, –CH=N), 7.98 (d, 2H, benzylidene), 7.89 (m, 1H, benzylidene), 7.76 (d, 2H, 4-hydroxyphenyl), 7.55 (s, 1H, phenylimino), 7.21 (d, 2H, phenylimino), 7.12 (d, 1H, phenylimino), 6.84 (d, 2H, 4-hydroxyphenyl) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.6$, 161.7, 160.5, 154.9, 143.4, 136.6, 135.8, 131.6, 129.0, 128.9, 128.5, 127.6, 122.9, 120.7, 116.3 ppm; MS (ES+, ToF): $m/z = 379$ ($[M + 1]^+$).

N'-[4-[(2-Nitrophenylimino)methyl]benzylidene]-4-hydroxybenzoic acid hydrazide (**35**, $C_{21}H_{16}N_4O_4$)
Yield: 65.6 %; TLC: $R_f = 0.38$; IR (KBr): $\bar{\nu} = 1{,}699$ (C=N str., CH=N), 1,620 (C=O str., secondary amide), 1,511 (asym. str., NO_2), 1,391 (C–O str. and O–H in plane bending, phenol), 828 (C–H out of plane bending, 1,4-disubstituted benzene ring), 754 (C–H out of plane bending, 1,2-disubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.22$ (s, 1H, –N=CH), 8.00 (d, 1H, phenylimino), 7.88 (d, 2H, benzylidene), 7.77 (m, 1H, benzylidene), 7.77 (m, 1H, phenylimino), 7.62 (d, 2H, 4-hydroxyphenyl), 7.53 (m, 2H, phenylimino), 6.64 (d, 2H, 4-hydroxyphenyl), 5.22 (s, 1H, –ArOH) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.9$, 161.8, 160.2, 148.8, 148.6, 143.7, 136.9, 136.3, 129.4, 128.8, 128.4, 128.4, 123.5, 122.4, 116.4 ppm; MS (ES+, ToF): $m/z = 389$ ($[M + 1]^+$).

N'-[4-[(4-Nitrophenylimino)methyl]benzylidene]-4-hydroxybenzoic acid hydrazide (**36**, $C_{21}H_{16}N_4O_4$)
Yield: 79.0 %; TLC: $R_f = 0.59$; IR (KBr): $\bar{\nu} = 1{,}695$ (C=N str., CH=N), 1,618 (C=O str., secondary amide), 1,506 (asym. str., NO_2), 1,345 (C–O str. and O–H in plane bending, phenol), 823 (C–H out of plane bending, 1,4-disubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.35$ (s, 1H, –N=CH), 8.06 (d, 2H, phenylimino), 7.99 (d, 2H, benzylidene), 7.89 (m, 1H, benzylidene), 7.70 (d, 2H, 4-hydroxyphenyl), 7.50 (d, 2H, phenylimino), 6.63 (d, 2H, 4-hydroxyphenyl) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.3$, 161.8, 160.4, 159.4, 146.8, 143.2, 136.4, 136.3, 129.7, 129.5, 129.5, 129.2, 128.8, 128.7, 126.6, 123.5, 123.4, 122.7, 122.6, 116.4, 116.3 ppm; MS (ES+, ToF): $m/z = 389$ ($[M + 1]^+$).

N'-[4-[(2-Chloro-4-nitrophenylimino)methyl]benzylidene]-4-hydroxybenzoic acid hydrazide (**37**, $C_{21}H_{15}ClN_4O_4$)
Yield: 61.4 %; TLC: $R_f = 0.51$; IR (KBr): $\bar{\nu} = 1{,}696$ (C=N str., CH=N), 1,619 (C=O str., secondary amide), 1,506 (asym. str., NO_2), 1,391 (C–O str. and O–H in plane bending, phenol), 874 (C–H out of plane bending, 1,2,4-trisubstituted benzene ring), 825 (C–H out of plane

bending, 1,4-disubstituted benzene ring), 745 (C–Cl str., Ar–Cl) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.48$ (s, 1H, –CH=N), 8.13 (s, 1H, phenylimino), 8.01 (d, 1H, phenylimino), 7.89 (d, 2H, benzylidene), 7.80 (m, 1H, benzylidene), 7.72 (d, 2H, 4-hydroxyphenyl), 7.62 (d, 1H, phenylimino), 6.65 (d, 2H, 4-hydroxyphenyl) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.6$, 161.6, 160.0, 149.7, 148.5, 143.5, 136.8, 129.3, 128.9, 128.9, 128.6, 125.3, 124.8, 120.9, 116.8 ppm; MS (ES+, ToF): *m/z* = 424 ([M + 1]$^+$).

N′-[4-[(3-Nitrophenylimino)methyl]benzylidene]-4-hydroxybenzoic acid hydrazide (**38**, $C_{21}H_{16}N_4O_4$)
Yield: 80.0 %; TLC: $R_f = 0.40$; IR (KBr): $\bar{\nu} = 1{,}698$ (C=N str., CH=N), 1,618 (C=O str., secondary amide), 1,524 (asym. str., NO_2), 1,389 (C–O str. and O–H in plane bending, phenol), 825 (C–H out of plane bending, 1,4-disubstituted benzene ring), 697 (C–C out of plane bending, 1,3-disubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.41$ (s, 1H, –N=CH), 8.23 (s, 1H, phenylimino), 8.22 (d, 1H, phenylimino), 7.89 (d, 2H, benzylidene), 7.84 (d, 1H, phenylimino), 7.80 (m, 1H, benzylidene), 7.72 (d, 2H, 4-hydroxyphenyl), 7.51 (m, 1H, phenylimino), 6.65 (d, 2H, 4-hydroxyphenyl), 5.23 (s, 1H, –ArOH) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.3$, 161.5, 160.2, 154.3, 149.8, 143.2, 136.6, 129.5, 129.4, 128.7, 128.6, 128.4, 119.9, 117.3, 116.9 ppm; MS (ES+, ToF): *m/z* = 389 ([M + 1]$^+$).

N′-[4-[(4-Chlorophenylimino)methyl]benzylidene]-4-hydroxybenzoic acid hydrazide (**39**, $C_{21}H_{16}ClN_3O_2$)
Yield: 82.7 %; TLC: $R_f = 0.42$; IR (KBr): $\bar{\nu} = 1{,}698$ (C=N str., CH=N), 1,618 (C=O str., secondary amide), 1,508 (C=C skeletal str., phenyl), 1,390 (C–O str. and O–H in plane bending, phenol), 826 (C–H out of plane bending, 1,4-disubstituted benzene ring), 730 (C–Cl str., Ar–Cl) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.10$ (s, 1H, CH=N), 7.91 (d, 2H, benzylidene), 7.89 (m, 1H, benzylidene), 7.76 (d, 2H, 4-hydroxyphenyl), 7.34 (d, 2H, phenylimino), 7.26 (d, 2H, phenylimino), 6.86 (d, 2H, 4-hydroxyphenyl), 5.50 (s, 1H, –ArOH) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.9$, 161.4, 160.4, 151.5, 143.8, 136.4, 132.6, 130.5, 130.4, 129.7, 128.9, 128.8, 123.9, 123.4, 116.5 ppm; MS (ES+, ToF): *m/z* = 379 ([M + 1]$^+$).

N′-[4-[(4-Chlorophenylimino)methyl]benzylidene]-2-hydroxybenzoic acid hydrazide (**40**, $C_{21}H_{16}ClN_3O_2$)
Yield: 70.5 %; TLC: $R_f = 0.60$; IR (KBr): $\bar{\nu} = 1{,}652$ (C=N str., CH=N), 1,619 (C=O str., secondary amide), 1,377 (C–O str. and O–H in plane bending, phenol), 827 (C–H out of plane bending, 1,4-disubstituted benzene ring), 738 (C–H out of plane bending, 1,2-disubstituted benzene ring), 691 (C–Cl str., Ar–Cl) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.44$ (s, 1H, –CH=N), 7.91 (d, 2H, benzylidene), 7.89 (m, 1H, benzylidene), 7.65 (d, 1H, 2-hydroxyphenyl), 7.35 (m, 1H, 2-hydroxyphenyl), 7.30 (d, 2H, phenylimino), 7.22 (d, 2H, phenylimino), 7.00 (m, 1H, 2-hydroxyphenyl), 6.89 (d, 1H, 2-hydroxyphenyl) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.2$, 160.6, 160.5, 159.0, 151.4, 143.4, 136.5, 133.7, 132.6, 130.5, 130.0, 129.6, 129.2, 123.9, 123.5, 121.8, 119.5 ppm; MS (ES+, ToF): *m/z* = 379 ([M + 1]$^+$).

N′-[4-[(3-Fluorophenylimino)methyl]benzylidene]-4-aminobenzoic acid hydrazide (**41**, $C_{21}H_{17}FN_4O$)
Yield: 62.3 %; TLC: $R_f = 0.48$; IR (KBr): $\bar{\nu} = 1{,}698$ (C=N str., CH=N), 1,618 (C=O str., secondary amide), 1,508 (C=C skeletal str., phenyl), 1,297 (C–N str., Ar–NH_2), 1,013 (C–F str., Ar–F), 828 (C–H out of plane bending, 1,4-disubstituted benzene ring), 698 (C–C out of plane bending, 1,3-disubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.44$ (s, 1H, –CH=N), 7.93 (d, 2H, benzylidene), 7.82 (d, 2H, benzylidene), 7.61 (d, 2H, 4-aminophenyl), 7.29 (s, 1H, phenylimino), 7.01 (d, 2H, phenylimino), 7.01 (m, 1H, phenylimino), 6.67 (d, 2H, 4-aminophenyl) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 164.4$, 163.4, 160.5, 154.9, 151.8, 143.9, 136.2, 131.6, 129.7, 128.3, 124.0, 117.7, 116.5, 114.3, 109.5 ppm; MS (ES+, ToF): *m/z* = 361 ([M + 1]$^+$).

N′-[4-[(3-Chloro-4-fluorophenylimino)methyl]benzylidene]-4-aminobenzoic acid hydrazide
(**42**, $C_{21}H_{16}ClFN_4O$)
Yield: 69.1 %; TLC: $R_f = 0.58$; IR (KBr): $\bar{\nu} = 1{,}696$ (C=N str., CH=N), 1,618 (C=O str., secondary amide), 1,503 (skeletal str., phenyl), 1,297 (C–N str., Ar–NH_2), 1,014 (C–F str., Ar–F), 827 (C–H out of plane bending, 1,4-disubstituted benzene ring), 728 (C–Cl str., Ar–Cl) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.12$ (s, 1H, –CH=N), 7.94 (d, 2H, benzylidene), 7.82 (d, 2H, benzylidene), 7.80 (d, 2H, 4-aminophenyl), 7.29 (s, 1H, phenylimino), 7.01 (d, 1H, phenylimino), 6.73 (d, 1H, phenylimino), 6.69 (d, 2H, 4-aminophenyl) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.7$, 161.5, 160.6, 151.6, 150.4, 143.7, 136.4, 129.9, 128.1, 124.4, 124.3, 122.3, 122.2, 118.3, 116.2 ppm; MS (ES+, ToF): *m/z* = 396 ([M + 1]$^+$).

N′-[4-[(Butylimino)methyl]benzylidene]-4-aminobenzoic acid hydrazide (**43**, $C_{19}H_{22}N_4O$)
Yield: 71.5 %; TLC: $R_f = 0.58$; IR (KBr): $\bar{\nu} = 1{,}697$ (C=N str., CH=N), 1,618 (C=O str., secondary amide), 1,509 (skeletal str., phenyl), 1,463 (CH_3 asym. bending, R–CH_3), 1,297 (C–N str., Ar–NH_2), 827 (C–H out of plane bending, 1,4-disubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.09$ (s, 1H, CH=N), 7.98 (d, 2H, benzylidene), 7.81 (d, 2H, benzylidene), 7.76 (d, 2H, 4-aminophenyl), 6.77 (d, 2H, 4-aminophenyl), 3.52 (t, 2H, –C_4H_9), 1.58 (m, 2H, –C_4H_9), 1.32 (m, 2H, –C_4H_9), 0.93 (t, 3H, –C_4H_9) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.9$, 160.3, 151.7, 143.5, 136.5, 129.8, 128.3, 124.1, 116.4, 55.9, 33.8, 20.1, 13.6 ppm; MS (ES+, ToF): *m/z* = 323 ([M + 1]$^+$).

N'-[4-[(Benzylimino)methyl]benzylidene]-4-aminobenzoic acid hydrazide (**44**, $C_{22}H_{20}N_4O$)

Yield: 63.3 %; TLC: $R_f = 0.62$; IR (KBr): $\bar{\nu} = 1{,}696$ (C=N str., CH=N), 1,618 (C=O str., secondary amide), 1,509 (skeletal str., phenyl), 1,297 (C–N str., Ar–NH_2), 828 (C–H out of plane bending, 1,4-disubstituted benzene ring), 731 (C–H out of plane bending, monosubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.18$ (s, 1H, –CH=N), 7.94 (d, 2H, benzylidene), 7.68 (d, 2H, benzylidene), 7.68 (d, 2H, phenylimino), 7.35 (d, 2H, 4-aminophenyl), 7.24 (d, 2H, phenylimino), 7.11 (m, 1H, phenylimino), 6.82 (d, 2H, 4-aminophenyl) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.6$, 160.4, 151.8, 143.6, 138.7, 136.6, 129.9, 129.3, 129.2, 128.8, 128.6, 128.5, 125.5, 124.0, 116.6, 58.5 ppm; MS (ES+, ToF): $m/z = 357$ ($[M + 1]^+$).

N'-[4-[(2-Hydroxyethylimino)methyl]benzylidene]-4-aminobenzoic acid hydrazide (**45**, $C_{17}H_{18}N_4O_2$)

Yield: 66.4 %; TLC: $R_f = 0.54$; IR (KBr): $\bar{\nu} = 1{,}667$ (C=N str., CH=N), 1,618 (C=O str., secondary amide), 1,510 (skeletal str., phenyl), 1,297 (C–N str., Ar–NH_2), 1,279 (C–O str. and O–H in plane bending, primary alcohol), 828 (C–H out of plane bending, 1,4-disubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.19$ (s, 1H, –CH=N), 7.96 (d, 2H, benzylidene), 7.81 (d, 2H, benzylidene), 7.78 (d, 2H, 4-aminophenyl), 6.67 (d, 2H, 4-aminophenyl), 3.86 (m, 2H, N-C_2H_4OH), 2.08 (t, 1H, –N–C_2H_4OH) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.7$, 160.4, 151.5, 143.6, 136.6, 129.7, 128.5, 124.3, 116.6, 64.3, 59.0 ppm; MS (ES+, ToF): $m/z = 311$ ($[M + 1]^+$).

N'-[4-[(Hydroxyimino)methyl]benzylidene]-4-aminobenzoic acid hydrazide (**46**, $C_{15}H_{14}N_4O_2$)

Yield: 64.2 %; TLC: $R_f = 0.54$; IR (KBr): $\bar{\nu} = 1{,}692$ (C=N str., CH=N), 1,618 (C=O str., secondary amide), 1,508 (C=C skeletal str., phenyl nucleus), 1,321 (C–N str., Ar–NH_2), 1,297 (C=O str. and O–H in-plane bending, primary alcohol), 829 (C–H out of plane bending, 1,4-disubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 8.01$ (s, 1H, –CH=N), 7.93 (d, 2H, benzylidene), 7.77 (d, 2H, benzylidene), 7.75 (d, 2H, 4-aminophenyl), 6.65 (d, 2H, 4-aminophenyl), 2.07 (s, 1H, –N–OH) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.6$, 151.1, 148.0, 143.7, 136.6, 136.4, 129.5, 129.4, 129.3, 129.3, 128.5, 124.1, 116.3 ppm; MS (ES+, ToF): $m/z = 283$ ($[M + 1]^+$).

N'-[4-[(Naphthalen-1-ylimino)methyl]benzylidene]-4-aminobenzoic acid hydrazide (**47**, $C_{25}H_{20}N_4O$)

Yield: 67.3 %; TLC: $R_f = 0.64$; IR (KBr): $\bar{\nu} = 1{,}696$ (C=N str., CH=N), 1,617 (C=O str., secondary amide), 1,514 (C=C skeletal str., phenyl nucleus), 1,321 (C–N str., Ar–NH_2), 826 (C–H out of plane bending, 1,4-disubstituted benzene ring), 771 (C–H out of plane bending, 1-naphthalenyl) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 7.99$ (s, 1H, –CH=N), 7.96 (d, 2H, benzylidene), 7.77 (d, 2H, naphthalene), 7.75 (d, 2H, benzylidene), 7.43 (d, 2H, 4-aminophenyl), 7.26 (m, 5H, naphthalene), 6.65 (d, 2H, 4-aminophenyl) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.5$, 160.3, 152.3, 151.2, 143.5, 136.4, 135.4, 129.9, 128.6, 128.5, 128.4, 127.7, 127.7, 126.5, 126.3, 126.2, 124.4, 116.4, 115.4 ppm; MS (ES+, ToF): $m/z = 393$ ($[M + 1]^+$).

N'-[4-[(Propylimino)methyl]benzylidene]-4-aminobenzoic acid hydrazide (**48**, $C_{18}H_{20}N_4O$)

Yield: 63.5 %; TLC: $R_f = 0.30$; IR (KBr): $\bar{\nu} = 1{,}696$ (C=N str., CH=N), 1,618 (C=O str., secondary amide), 1,511 (C=C skeletal str., phenyl nucleus), 1,464 (CH_3 asym. bending, R–CH_3), 1,276 (C–N str., Ar–NH_2), 827 (C–H out of plane bending, 1,4-disubstituted benzene ring) cm^{-1}; ^{1}H NMR (MeOH-d_4): $\delta = 7.95$ (d, 2H, benzylidene), 7.93 (s, 1H, –CH=N), 7.45 (d, 2H, benzylidene), 7.32 (d, 2H, 4-aminophenyl), 6.85 (d, 2H, 4-aminophenyl), 3.66 (t, 2H, –C_3H_7), 0.94 (t, 3H, –C_3H_7) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.7$, 160.4, 151.6, 143.5, 136.5, 129.7, 128.5, 124.2, 116.3, 58.5, 24.9, 11.4 ppm; MS (ES+, ToF): $m/z = 309$ ($[M + 1]^+$).

Evaluation of antimicrobial activity: determination of MIC

The antimicrobial activity of the synthesized compounds was evaluated against Gram-positive bacteria *Staphylococcus aureus* MTCC 2901 and *Bacillus subtilis* MTCC 2063, the Gram-negative bacterium *Escherichia coli* MTCC 1652, and fungal strains *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 8189, using the tube-dilution method [19]. Dilutions of test and standard compounds were prepared in double-strength nutrient broth I.P. (bacteria) or Sabouraud dextrose broth I.P. (fungi) [43]. The samples were incubated at 37 °C for 24 h (bacteria), at 25 °C for 7 days (*A. niger*), or at 37 °C for 48 h (*C. albicans*) and the results were recorded in terms of MIC.

Determination of MBC/MFC

The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by subculturing 100 mm^3 of culture from each tube (which remained clear in the MIC determination) on fresh medium. MBC and MFC values are the lowest concentrations of compound that produce a 99.9 % end point reduction [44].

Antiviral assays

The antiviral assays (except anti-human immunodeficiency virus (HIV) assay) were based on inhibition of virus-induced cytopathicity in CRFK (feline corona virus and feline herpes virus), HEL (herpes simplex virus type 1 (HSV-1), HSV-2 (G), vaccinia virus, and vesicular stomatitis virus), Vero (parainfluenza-3, reovirus-1, Sindbis, Coxsackie B4, and Punta Toro virus), and HeLa (vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus) cell cultures. Confluent cell cultures in microtiter 96-well plates were inoculated with 100 cell culture inhibitory dose-50 ($CCID_{50}$) of the virus (1 $CCID_{50}$, being the virus dose infecting 50 % of the cell cultures) in the presence of different concentrations (100, 20, 4, … μg/cm^3) 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.

Evaluation of anti-HIV activity

The anti-HIV activity and cytotoxicity were evaluated against HIV-1 strain IIIB and HIV-2 strain ROD in MT-4 cell cultures by use of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Briefly, virus stocks were titrated in MT-4 cells and expressed as the 50 % cell culture infective dose ($CCID_{50}$). MT-4 cells were suspended in culture medium at 1×10^5 cells/cm^3 and infected with HIV at a multiplicity of infection of 0.02. Immediately after viral infection, 100 mm^3 of the cell suspension was placed in wells of a flat-bottomed microtiter tray containing different concentrations of the test compounds. After incubation for four days at 37 °C, the number of viable cells was determined by use of the MTT method. Compounds were tested in parallel for cytotoxic effects in uninfected MT-4 cells.

Evaluation of anticancer activity

The anticancer activity of compounds **1–48** was determined against human colon (HCT116) and breast (MCF7) cancer cell lines. All cell lines were cultured in RPMI 1640 (Sigma) supplemented with 10 % heat-inactivated fetal bovine serum (FBS) (PAA Laboratories) and 1 % penicillin/streptomycin (PAA Laboratories). Cultures were maintained in a humidified incubator at 37 °C in an atmosphere of 5 % CO_2. Anticancer activity of synthesized compounds at different concentrations was assessed by use of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma) assay, as described by Mosmann [22], but with minor modification, after incubation for 72 h. Assay plates were read by use of a spectrophotometer at 520 nm. Data generated were used to plot a dose–response curve from which concentrations of the test compounds required to kill 50 % of cell population (IC_{50}) were determined. Anticancer activity was expressed as the mean IC_{50} from three independent experiments.

QSAR studies

The structures of **1–48** were first pre-optimized by use of MM^+ procedure included in Hyperchem 6.03 [45] and the resulting geometries were further refined by means of the semiempirical method PM3 (Parametric Method-3). We chose a gradient norm limit of 0.04 kJ/Å for the geometry optimization. The lowest energy structure was used for each molecule to calculate physicochemical properties using TSAR 3.3 software for Windows [46]. Further, the regression analysis was performed by use of the SPSS software package [47].

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