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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, characterization, computational studies, and pharmacological evaluation of substituted-*N*′-[(1E) substituted-phenylmethylidene]benzohydrazide analogs

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Abstract A series of substituted-*N*′-[(1E)-substituted-phenylmethylidene]benzohydrazide analogs were synthesized and authenticated by TLC, UV–Visible, FTIR, and NMR spectroscopic techniques. The physicochemical similarity of the new analogs with standard drugs was assessed by calculating from a set of ten physicochemical properties using software programs. The information so obtained can be related to prediction of biological activity for important targets. All the target compounds **4a–n** were evaluated for their antioxidant, anti-inflammatory, and antimicrobial activity using different in vitro models. The test compounds demonstrated good similarity values with respect to the standard drugs. The compounds **4c**, **4d,** and **4e** have emerged as important lead compounds showing potential anti-inflammatory; and **4b**, **4f**, and **4c** having antioxidant profile. While studying MIC against bacterial strains **4c**, **4f**, **4i**, **4k,** and **4m** were most active among all the target compounds. All compounds were found to have very good antifungal activity. The compounds having nitro substitution at the arylidene moiety i.e., **4c** and **4f** showed the most potent antifungal as well as antibacterial activities. While studying total antioxidant activity, all target compounds were found to have good antioxidant activity.

Keywords 4-Chlorobenzoic acid · *o*-Benzoylbenzoic acid · Hydrazone · Antioxidant · Anti-inflammatory activity · Antimicrobial activity

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Introduction

Hydrazone is a versatile moiety that exhibits a wide variety of biological activities. A hydrazone is a class of organic compounds with the structure $R_1R_2C{=}NNH_2$. They are related to ketones and aldehydes by the replacement of the oxygen with the $–NNH_2$ functional group. Hydrazones are formed usually by the action of hydrazine on ketones or aldehydes. A hydrazone is an intermediate in the Wolff–Kishner reduction (Corey, 1976; Corey and Enders, 1976).

Hydrazone compounds are not only intermediates but they are also very effective organic compounds. There are many clinically effective hydrazide–hydrazones such as nifuroxazide (intestinal antiseptic), iproniazide, and isocarboxazide (antitubercular) (Rollas and Kucukguzel, 2007). The most significant reactivity of the hydrazones is their nucleophilicity. Thus, reactions like Mannich reaction, coupling reaction, and halogenations have taken place readily at such carbon. It was reported that hard nucleophiles attack preferentially nitrogen atom, while soft ones attack at carbon atom (Atlan *et al.*, 2000).

Non-steroidal anti-inflammatory drugs (NSAIDs) exerts their effects by inhibition of prostaglandin production. The pharmacological target of NSAIDs is cyclooxygenase (COX), which catalyzes the first committed step in arachidonic acid metabolism. It was reported that crystal structures of COX enzymes with carboxylic acid containing NSAIDs shows that the inhibitors are positioned in a similar fashion with their

carboxylates co-ordinates to Arg-120 and their aromatic functionality projecting into the cyclooxygenase active site toward Tyr-385. These studies suggest that the presence of hydrazone moiety in some compounds have such a pharmacophoric effect which leads to inhibition of COX (Eissa *et al.*, 2009; Singh *et al.*, 1992; Elassar *et al.*, 2007; Kurumbail *et al.*, 1996). Mizushima and Kobayashi, 1968 have reported protein denaturation as in vitro screening model for determining anti-inflammatory activity of compounds (Mizushima and Kobayashi, 1968).

Hydrazones are also found to have considerable antioxidant activity and can be expressed as total antioxidant activity and hydrogen peroxide scavenging activity. Oxidative stress is a well-known mechanism that is responsible for the development of vascular damage. Reactive oxygen species (ROS) are normally produced throughout oxygen metabolism and play a major role in physiological and pathological cell redox signaling. Oxidative stress results from an imbalance due to overproduction of ROS by the different cellular sources. Antioxidants inhibit the generation of ROS and the subsequent formation of lipid peroxidation products, thereby preventing both oxidative and carbonyl stress. Most antioxidants prevent LDL oxidation in cell-dependent and cell-free systems (Picot *et al.*, 1994; Sreenivasa *et al.*, 2009; Melnyk *et al.*, 2006; Belkheiri *et al.*, 2010; Sies, 1991; Stocker, 1999; Rao *et al.*, 2005; Lingnert *et al.*, 1979; Chanda and Dave, 2009).

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 such as methicillin-resistant *Staphylococcus aureus* and *Staphylococcus* epidermis and vancomycin-resistant *Enterococcus* is a problem of ever-increasing significance (Sriram *et al.*, 2010; Mohamed *et al.*, 2007). There are two ways to counterbalance this challenge: one is the controlled use of the currently marketed antibiotics and the other is the development of novel antimicrobial agents.

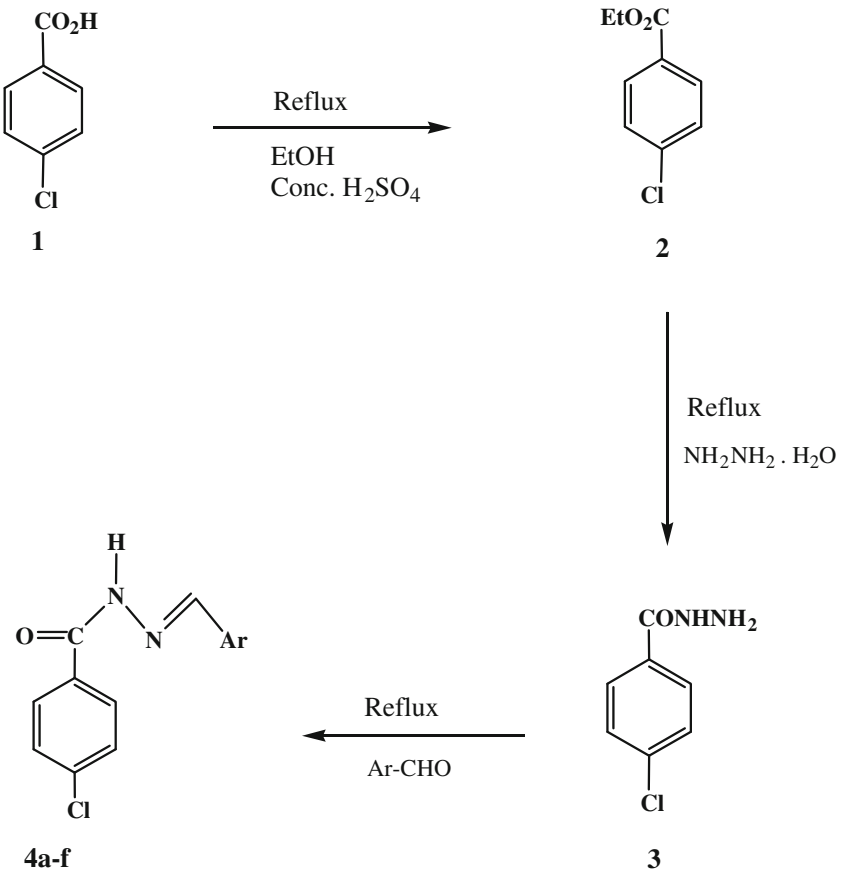
Material and method

Experimental

Melting points of newly synthesized compounds were determined on digital melting point apparatus (Flora; Perfit India) and were found uncorrected. Silica gel G plates of 3 × 15 cm were used for TLC and spots were located by iodine chamber (Table 1). The structures of the synthesized compounds were confirmed by spectral data. The λ_{max} was calculated using double beam UV–Visible 1800 Shimadzu spectrophotometer. The IR spectra were recorded on FTIR-Shimadzu spectrometer using Nujol method. ^{1}H NMR spectra were recorded on BRUKER AVANCE II 400 NMR spectrometer using DMSO as solvent and TMS as internal standard, values were expressed in δ ppm. Structural similarity studies were performed using Chem 3D (2012) (Nikolova and Jaworska 2003).

Table 1 Physical data of synthesized compounds

Compounds	Molecular formula	% Age yield	Molecular weight	Solubility	Melting point (°C)	λ_{max}	R_f value
2	$C_9H_9ClO_2$	99.70	184.623	EtOH, MeOH	40.00	239	0.65
3	$C_7H_7ClN_2O$	93.20	170.599	EtOH, MeOH	213.00	237	0.37
4a	$C_{14}H_{11}ClN_2O_2$	99.90	274.708	DMSO, MeOH, $CHCl_3$	240.00	243	0.54
4b	$C_{15}H_{13}ClN_2O_3$	90.00	304.734	DMSO, MeOH, $CHCl_3$	241.00	248	0.42
4c	$C_{14}H_{10}ClN_3O_3$	99.80	303.706	DMSO, MeOH, $CHCl_3$	235.00	255	0.47
4d	$C_{15}H_{13}ClN_2O$	99.80	272.735	DMSO, MeOH, $CHCl_3$	233.00	240	0.68
4e	$C_{18}H_{13}ClN_2O_2$	99.80	324.768	DMSO, MeOH, $CHCl_3$	270.00	276	0.40
4f	$C_{14}H_{10}ClN_3O_3$	99.80	303.706	DMSO, MeOH, $CHCl_3$	220.00	244	0.35
6	$C_{14}H_{10}O_3$	98.7	226.233	EtOH	128.00	240	0.55
7	$C_{16}H_{14}O_3$	80.00	254.287	EtOH, MeOH	48.00	246	0.67
8	$C_{14}H_{12}N_2O_2$	95.60	240.263	MeOH	299.00	242	0.76
4g	$C_{21}H_{16}N_2O_2$	90.40	328.372	DMSO, $CHCl_3$	256.00	248	0.56
4h	$C_{21}H_{15}ClN_2O_2$	94.90	362.817	DMSO, $CHCl_3$	278.00	249	0.45
4i	$C_{21}H_{15}N_3O_4$	84.80	373.37	DMSO, $CHCl_3$	259.00	257	0.43
4j	$C_{22}H_{18}N_2O_2$	98.90	342.399	DMSO, $CHCl_3$	266.00	246	0.38
4k	$C_{22}H_{18}N_2O_4$	99.00	374.398	DMSO, $CHCl_3$	270.00	247	0.53
4l	$C_{21}H_{16}N_2O_3$	99.00	344.372	DMSO, $CHCl_3$	265.00	250	0.42
4m	$C_{21}H_{15}N_3O_4$	80.00	373.37	DMSO, $CHCl_3$	270.00	252	0.31
4n	$C_{21}H_{16}N_2O_3$	71.33	344.372	DMSO, $CHCl_3$	273.00	250	0.53



Scheme 1 Synthesis of 4-chloro-*N*'-[(1E)-substituted-phenylmethylidene]benzohydrazide analogs (**4a–f**)

General methods

A series of 4-chlorobenzoic acid and *o*-benzoylbenzoic acid hydrazone analogs were synthesized as per the Schemes 1 and 2. Fischer esterification of substituted benzoic acid with methanol in the presence of concentrated sulfuric acid yielded the corresponding ester which on further reaction with hydrazine hydrate in ethanol afforded the corresponding 4-chlorobenzoic acid hydrazide. The hydrazide so formed was further reacted with aromatic aldehydes in the presence of few drops of glacial acetic acid to yield the 4-chloro-*N*′-[(1E)-substituted-phenylmethylidene]benzohydrazide (**4a–f**), *o*-benzoyl-*N*′-[(1E)-substituted-phenylmethylidene]benzohydrazide(**4g–n**). The synthesized substituted-*N*′-[(1E)-substituted phenyl methylidene]benzohydrazide were characterized on the basis of the spectral and analytical studies (Rajput and Rajput, 2009; Sunil *et al.*, 2010).

*General procedure for synthesis of ethyl-4-chlorobenzoate, (**2**)*

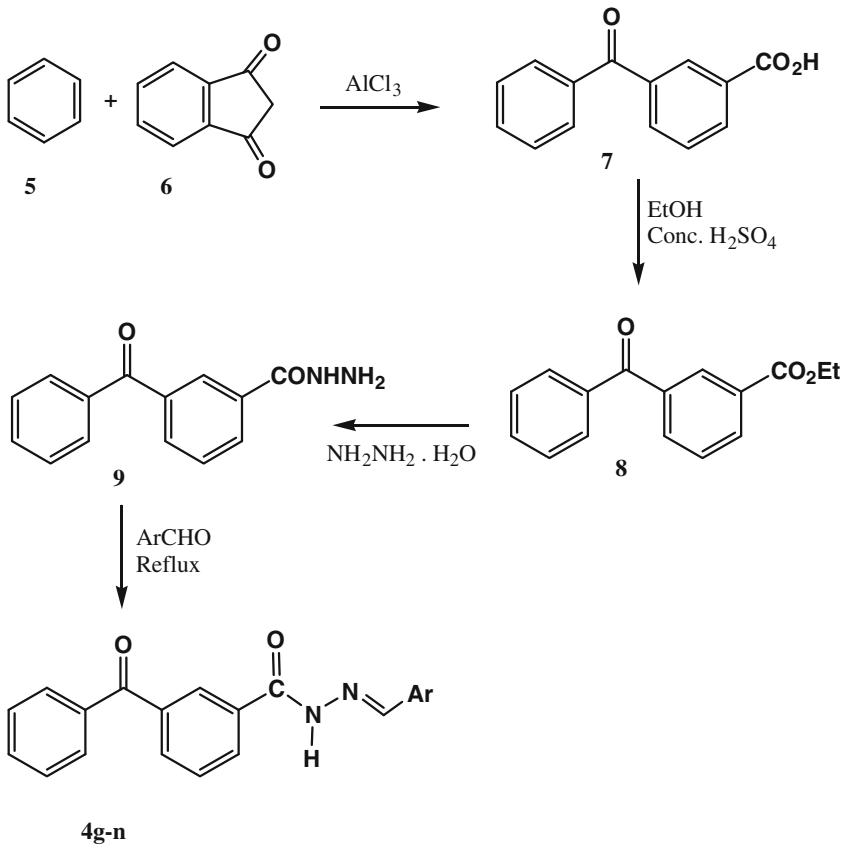
4-Chlorobenzoic acid (0.05 mol) was dissolved in ethanol (10 ml) along with 4–5 drops of concentrated sulfuric acid in a round bottom flask. Contents were refluxed for 40 min, cooled, and added chilled water. Excess of acid was neutralized by addition of saturated sodium bicarbonate solution. The mixture was cooled and ester was filtered out in good yield (Lingnert *et al.*, 1979).

*General procedure for the synthesis of 4-chlorobenzohydrazide, (**3**)*

Hydrazide was prepared by refluxing hydrazine hydrate (0.05 mol) with ethyl-4-chlorobenzoate (0.05 mol) in ethanol (5.0 ml) at 80 °C for 4 h. Crystals were separated out by filtration on cooling and can be recrystallized from ethanol.

*General procedure for the synthesis of 4-chloro-N'-[(1E)-substituted-phenylmethylidene]benzohydrazides, (**4a–f**)*

4-Chloro benzohydrazide **3** (0.0025 mol) was dissolved in methanol, added (0.0025 mol) substituted aromatic aldehyde and three drops of glacial acetic acid, mixture was refluxed for 30 min. The crude product was recrystallized by a mixture of cold water and alcohol.



Scheme 2 Synthesis of *o*-benzoyl-*N*'-[(1E)-substituted-phenylmethylidene]benzohydrazide analogs (**4g–n**)

*General procedure for the synthesis of o-benzoylbenzoic acid, (**6**)*

Anhydrous aluminum chloride (0.15 M) was suspended in benzene **5** (0.51 M) in round bottom flask with a provision for reflux condenser. To the mixture added phthalic anhydride **6** (0.07 M) in small proportions with constant shaking. The reaction mixture was slightly warmed after addition of first installment of phthalic anhydride and cooled on ice bath when became vigorous. After the addition of phthalic anhydride the reaction mixture was refluxed for 3 h under anhydrous conditions ($CaCl_2$ tube was attached to the end of the condenser) until the evolution of hydrochloride gas ceased. Contents of flask were cooled and to this ice cold 2.5 % hydrochloric acid was added. Reaction mixture was subjected to steam distillation to remove unreacted benzene. Reaction mixture was filtered out while hot, cooled, and crude *o*-benzoylbenzoic acid was separated out. Crude product was dissolved in anhydrous sodium carbonate solution, filtered and acidified with hydrochloric acid to separate out the *o*-benzoylbenzoic acid in pure form.

*General procedure for synthesis of ethyl-o-benzoylbenzoate, (**8**)*

4-Benzoylbenzoic acid (0.05 mol) was dissolved in ethanol (10 ml) along with 4–5 drops of concentrated sulfuric acid in a round bottom flask. Contents were refluxed for 40 min. Cooled and chilled water was added. Excess of acid was neutralized by addition of saturated sodium bicarbonate solution. The mixture was cooled, and ester was filtered out in good yield.

*General procedure for the synthesis of o-benzoylbenzohydrazide, (**9**)*

Hydrazide was prepared by refluxing hydrazine hydrate (0.05 M) with ethyl-*o*-benzoylbenzoate **7** (0.05 M) in ethanol (5.0 ml) at 80 °C for 4 h. The reaction was monitored by TLC. The white colored crystals were separated out by filtration on cooling and can be recrystallized from ethanol.

*General procedures for the synthesis of o-benzoyl-N′-[(1E)-substituted-phenylmethylidene]benzohydrazides, (**4g–n**)*

o-Benzoylbenzohydrazide **8** (0.0025 M) was dissolved in methanol. In this substituted aromatic aldehyde (0.0025 M) with three drops of glacial acetic acid was added. Mixture was refluxed for 4–5 h. The reaction was monitored by TLC. The white-colored crude product was filtered out and recrystallized by a mixture of cold water and ethanol.

Spectral data

Ethyl-4-chlorobenzoate IR: 3034.03, 1726.29, 1421.5, 837.11, 756.10 cm^{-1}; ^1H NMR (DMSO): 7.91 (d, 2H of Ph), 7.38 (d, 2H of Ph), 4.29 (q, 2H of CH_2), 1.30 (t, 3H of CH_3).

4-Chlorobenzohydrazide IR: 3277.06, 3159.40, 1456.21, 866.04, 738.74 cm^{-1}; ^1H NMR (DMSO): 9.9 (s, NH), 7.8-7.9 (d, 2H of Ph), 7.4-7.6 (d, 2H of Ph), 4.6 (s, of NH_2).

*4-Chloro-N′-[(1E)-2-hydroxyphenylmethylidene]benzohydrazides, (**4a**)* IR: 3398.57, 3232.70, 3072.60, 1680.70, 1638.00, 1600.92, 860.25, 636.51 cm^{-1}; ^1H NMR (DMSO): δ 7.9 (s, of NH), 7.8 (d, 2H of Ph), 7.7 (d, 2H of Ph), 7.5 (d, 2H of Ph), 7.37 (d, 2H of Ph), 7.3 (s, CH), MS: *m/z* 274.05, 275.05 (M + 1), 276.05 (M + 2).

*4-Chloro-N′-[(1E)-4-hydroxy-3-methoxyphenylmethylidene]benzohydrazides, (**4b**)* IR: 3387.80, 3250.05, 3014.74, 2912.00, 1409.00, 1620.21, 1670.00, 1577.77, 1276.00, 854.57, 611.00 cm^{-1}; ^1H NMR (DMSO): δ 8.9 (s, of CH), 7.9 (s, of NH), 7.5 (d, 2H of Ph), 7.4 (s, OH), 7.3 (d, 2H of Ph), 7.0 (d, 2H of Ph), 3.42 (s, of OCH_3); MS: *m/z* 274.05, 275.05 (M + 1), 276.05 (M + 2).

*4-Chloro-N′-[(1E)-4-nitrophenylmethylidene]benzohydrazides, (**4c**)* IR: 3381.21, 3059.10, 1649.14, 1620.00, 1548.84, 1578.00, 821.68, 707.88 cm^{-1}; ^1H NMR (DMSO): δ 8.1 (s, of NH), 7.98 (d, 2H of Ph), 7.96 (d, 2H of Ph), 7.94 (d, 2H of Ph), 7.7 (d, 2H of Ph), 7.1 (s, CH); MS: *m/z* 303.04, 304.04 (M + 1), 305.04 (M + 2).

*4-Chloro-N′-[(1E)-4-methylphenylmethylidene]benzohydrazides, (**4d**)* IR: 3402.43, 3107.00, 2974.00, 1629.85, 1627.00, 1563.00, 1344.00, 821.68, 726.00 cm^{-1}; ^1H NMR (DMSO): δ 7.8 (s, of NH), 7.7 (d, 2H of Ph), 7.6 (d, 2H of Ph), 7.45 (d, 2H of Ph), 7.4 (d, 2H of Ph), 7.3 (s, CH), 2.4 (s, of CH_3); MS: *m/z* 272.07, 273.07 (M + 1), 274.07 (M + 2).

*4-Chloro-N′-[(1E)-2-hydroxy-naphthalen-1-ylmethylene)-hydrazide, (**4e**)* IR: 3290.00, 3200.00, 2978.09, 1606.70, 1650.00, 1554.44, 937.40, 611.00 cm^{-1}; ^1H NMR (DMSO): δ 8.0 (s, NH), 7.89 (d, 2H of Ph), 7.86 (d, 2H of Ph), 7.63 (m, 1Hof Ph), 7.45 (d, 2H of Ph), 7.30 (m, 1H of Ph), 7.21 (m, 1H of Ph), 6.8 (s, CH), 5.0 (s, OH); MS: *m/z* 324.05, 325.05 (M + 1), 326.05 (M + 2).

*4-Chloro-N′-[(1E)-3-nitro-benzylidene)-hydrazide, (**4f**)* IR: 3201.63, 2953.00, 1651.00, 1665.00, 1529.55, 1558.00, 921.97, 663.00 cm^{-1};^1H NMR (DMSO): δ 8.4 (d, 2H of Ph), 8.2 (d, 2H of Ph), 8.0 (s, of NH), 7.89 (d, 2H of Ph), 7.60 (m, 1H of Ph), 7.45 (d, 2H of Ph), 7.0 (s, CH); MS: *m/z* 303.04, 304.04 (M + 1), 305.04 (M + 2).

*o-Benzoylbenzoic acid, (**7**)* IR: 3360.00, 2930.00, 1680.00, 1530.00, 930.00; ^1H NMR (DMSO): δ 11 (s, 1H, OH), 8.43 (d, 1H, Ar–H), 8.18 (d, 1H, Ar–H), 7.95 (d, 1H, Ar–H), 7.80 (t, 1H, Ar–H), 7.63 (d, 2H, Ar–H), 7.61 (t, 1H, Ar–H), 7.51 (t, 2H, Ar–H).

*Ethyl-o-benzoylbenzoate, (**8**)* IR: 3035.96, 1735.93, 1570.00, 1271.00, 947.05; ^1H NMR (DMSO): δ 8.33 (d, 1H, Ar–H), 8.02 (d, 1H, Ar–H), 7.82 (t, 1H, Ar–H), 7.70 (t, 1H, Ar–H), 7.64 (d, 2H, Ar–H), 7.62 (t, 1H, Ar–H), 7.52 (t, 2H, Ar–H), 4.31 (q, 2H, CH_2), 1.30 (t, 3H, CH_3).

*o-Benzoylbenzohydrazide, (**9**)* IR: 3487.00, 3053.32, 1541.00, 1843.00, 790.81; ^1H NMR (DMSO): δ 8.40 (d, 1H, Ar–H), 7.86 (t, 1H, Ar–H), 7.79 (t, 1H, Ar–H), 7.77 (d, 1H, Ar–H), 7.65 (d, 2H, Ar–H), 7.64 (t, 1H, Ar–H), 7.53 (t, 2H, Ar–H), 6.85 (s, 1H, of NH), 3.34 (s, 1H, of NH_2), 1.78 (s, 1H, of NH_2).

*o-Benzoyl-N'-[(1E)-phenylmethylidene]benzohydrazide, (**4g**)* IR: 3331.00, 3072.00, 1635.64, 1630.00, 1581.63, 817.82;^1H NMR (DMSO): δ 12.50 (s, 1H, –CONH), 8.40 (d, 1H, Ar–H), 7.93 (d, 2H, Ar–H), 7.86 (t, 1H, Ar–H), 7.79 (m, 1H, Ar–H), 7.78 (m, 1H, Ar–H), 7.63 (d, 2H, Ar–H), 7.61 (t, 1H, Ar–H), 7.58 (m, 3H, Ar–H), 7.52 (m, 2H, Ar–H), 7.37 (s, 1H, CH), MS: *m/z* 328.12, 329.12 (M + 1), 330.13 (M + 2).

*o-Benzoyl-N'-[(1E)-4-chlorophenylmethylidene]benzohydrazide, (**4h**)* IR: 3282.84, 3035.96, 1664.57, 1644.00, 1521.84, 885.33, 671.23; ^1H NMR (DMSO): δ 11.5 (s, 1H, –CONH), 8.40 (d, 1H, Ar–H), 7.86 (t, 1H, Ar–H), 7.82 (d, 2H, Ar–H), 7.80 (t, 1H, Ar–H), 7.64 (m, 2H, Ar–H), 7.61 (m, 1H, Ar–H), 7.53 (t, 2H, Ar–H), 7.47 (d, 2H, Ar–H), 7.37(d, 1H, Ar–H); MS: *m/z* 362.08, 363.09 (M + 1), 364.08 (M + 2).

*o-Benzoyl-N'-[(1E)-4-nitrophenylmethylidene]benzohydrazide, (**4i**)* IR: 3429.00, 3072.00, 1655.00, 1633.71, 1556.50,

1519.00, 1049.00; ^{1}H NMR (DMSO): δ 11.5 (s, 1H, –CONH), 8.48 (d, 1H, Ar–H), 8.27 (d, 2H, Ar–H), 7.97 (d, 2H, Ar–H), 7.86 (t, 1H, Ar–H), 7.79 (t, 1H, Ar–H), 7.76 (d, 1H, Ar–H), 7.65 (m, 2H, Ar–H), 7.63 (m, 1H, Ar–H), 7.51 (t, 2H, Ar–H), 7.30 (s, 1H, CH); MS: *m/z* 373.11, 374.11 (M + 1), 375.11 (M + 2).

*o-Benzoyl-N'-[(1E)-4-methylphenylmethylidene]benzohydrazide, (**4j**)* IR: 3311.00, 3018.00, 2792.93, 1676.00, 1668.00, 1560.00, 898.93; ^{1}H NMR (DMSO): δ 11.80 (s, 1H, –CONH), 8.38 (d, 1H, Ar–H), 7.91 (d, 2H, Ar–H), 7.86 (t, 1H, Ar–H), 7.79 (m, 1H, Ar–H), 7.77 (m, 1H, Ar–H), 7.63 (m, 2H, Ar–H), 7.61 (m, 1H, Ar–H), 7.51 (t, 2H, Ar–H), 7.40 (d, 2H, Ar–H), 7.37 (s, 1H, CH), 2.41 (s, 3H, CH_3); MS: *m/z* 342.14, 343.14 (M + 1), 344.14 (M + 2).

*o-Benzoyl-N'-[(1E)-4-hydroxy-3-methoxyphenylmethylide-ne]benzohydrazide, (**4k**)* IR: 3427.00, 3327.00, 3070.00, 2912.00, 1637.00, 1558.48, 1535.00, 1296.00, 916.00; ^{1}H NMR (DMSO): δ 11.60 (s, 1H, –CONH), 8.37 (d, 1H, Ar–H), 7.93 (s, 1H, CH), 7.84 (m, 1H, Ar–H), 7.81 (m, 1H, Ar–H), 7.76 (d, 1H, Ar–H), 7.64 (m, 2H, Ar–H), 7.62 (m, 1H, Ar–H), 7.53 (t, 2H, Ar–H), 7.37 (m, 1H, Ar–H), 7.34 (m, 1H, Ar–H), 6.88 (d, 1H, Ar–H), 5.79 (s, 1H, OH), 3.83 (s, 3H, CH_3); MS: *m/z* 344.13, 345.13 (M + 1), 346.13 (M + 2).

*o-Benzoyl-N'-[(1E)-4-hydroxyphenylmethylidene]benzohy-drazide, (**4l**)* IR: 3280.00, 3179.00, 3051.00, 1629.85, 1529.00, 1485.00, 810.00; ^{1}H NMR (DMSO): δ 12.2 (s, 1H, –CONH), 8.47 (d, 1H, Ar–H), 7.93 (s, 1H, CH), 7.86 (t, 1H, Ar–H), 7.78 (m, 1H, Ar–H), 7.75 (m, 1H, Ar–H), 7.67 (m, 2H, Ar–H), 7.65 (m, 2H, -CH), 7.62 (m, 1H, Ar–H), 7.54 (t, 2H, Ar–H), 6.85 (d, 2H, Ar–H), 4.98 (s, 1H, OH); MS: *m/z* 344.12, 345.12 (M + 1), 346.12 (M + 2).

*o-Benzoyl-N'-[(1E)-2-nitrophenylmethylidene]benzohydrazide, (**4m**)* IR: 3361.00, 2984.00, 1660.70, 1654.00, 1465.26, 1359.82, 910.40; ^{1}H NMR (DMSO): δ 12.4 (s, 1H, –CONH), 8.4 (d, 1H, Ar–H), 8.08 (d, 1H, Ar–H), 7.95 (d, 1H, Ar–H), 7.9 (s, 1H, CH), 7.86 (t, 1H, Ar–H), 7.79 (m, 1H, Ar–H), 7.77 (m, 1H, Ar–H), 7.69 (t, 1H, Ar–H), 7.64 (m, 2H, Ar–H), 7.62 (m, 1H, Ar–H), 7.59 (m, 1H, Ar–H), 7.51 (t, 2H, Ar–H); MS: *m/z* 373.11, 374.11 (M + 1), 375.11 (M + 2).

*o-Benzoyl-N'-[(1E)-2-hydroxyphenylmethylidene]benzohy-drazide, (**4n**)* IR: 3300.00, 3267.00, 3010.00, 1649.85, 1539.00, 1490.00, 860; ^{1}H NMR (DMSO): δ 10.7 (s, 1H, –CONH), 8.42 (d, 1H, Ar–H), 8.34 (s, 1H, CH), 7.86 (t, 1H, Ar–H), 7.79 (m, 1H, Ar–H), 7.75 (m, 1H, Ar–H), 7.68 (m, 2H, Ar–H), 7.65 (m, 1H, Ar–H), 7.53 (t, 3H, Ar–H), 7.32 (t, 1H, Ar–H), 6.93 (m, 1H, Ar–H), 6.91 (m, 1H, Ar–H), 5.18 (s, 1H, OH); MS: *m/z* 344.12, 345.12 (M + 1), 346.12 (M + 2).

Similarity studies

Assessments of structural similarity of test compounds **4a–n** were compared to that of standard compounds. Assessment of structural similarity studies was performed by means of physico-chemical and steric similarity between the standard drugs and new analogs designed. These studies helped us to determine good spatial compatibility and effective binding to enzymatic site as lock and key model. The information was used for prediction of biological activity of important target compounds. Therefore, we calculated a number of parameters for the test compounds using Chem 3D and compared them to the values obtained for standard compounds.

The standard drug used for assessment of similarity with target compounds were taken from the literature as: nifuroxazide (intestinal antiseptic), tosufloxacin tosylate, pyridoxal salicyloyl hydrazone, and cefixime (anti-microbial agent). Various set of parameters were used for calculations as given in Table 2. The distance d_i of a particular target compound i can be presented as

$$\mathrm{d}i^2 = \sum_{j=1}^{n} \frac{\left(1 - X_{i,j}/X_{i,\mathrm{standard}}\right)^2}{n}$$

where $X_{i,j}$ is value of molecular parameters i for compound j; $X_{i,\mathrm{standard}}$ is the value of the same molecular parameter i for standard drug; n is the total number of the considered molecular parameters.

The similarity of the compounds can be calculated as:

$$\%\,\mathrm{Age\ similarity} = (1 - R) \times 100$$

where R is quadratic mean also known as the root mean square and R can be calculated as:

$$R = \sqrt{\mathrm{d}i^2}$$

Assessment of structural similarities of target compounds with standard drugs showed that compound **4a**, **4b**, **4c**, **4d**, **and 4f** has good % age similarity except **4e** (Table 3).

Quantitative structure–activity relationship-QSAR

The term quantitative structure–activity relationship (QSAR) implies the empirical relationships that use molecular parameters to quantify a pharmacological or chemical property for a set of molecules.

The logarithm of the octanol/water partition coefficient has been widely used as the sole physicochemical property

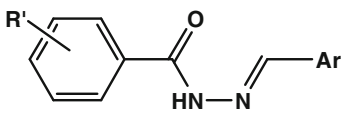
Table 2 Calculation of various molecular properties of target compounds

Compounds	SAS (Å^3)	MS (Å^3)	CSEV (Å^3)	Ovality	MR (cm^3/mol)	MTI	WI	BI	MW	Log *P*
4a	486.988	249.021	198.182	1.5148	7.6716	5,893	826	207,525	274.708	3.532
4b	535.536	276.697	222.644	1.5591	8.2885	7,684	1,104	337,123	304.734	3.406
4c	529.718	275.376	225.459	1.5372	8.3074	8,014	1,142	348,452	303.706	3.133
4d	512.973	263.596	210.681	1.5394	7.9823	6,277	850	213,402	272.735	4.409
4e	529.190	286.118	241.515	1.5255	9.3596	9,625	1,324	377,501	324.768	4.530
4f	537.939	276.885	221.998	1.5616	8.3074	7,783	1,106	337,752	303.707	3.133
4g	586.111	313.857	266.645	1.5666	10.037	12,737	1,654	552,411	328.372	4.573
4h	609.866	328.62	280.869	1.5844	10.529	13,772	1,817	674,005	362.817	5.132
4i	637.035	344.787	296.904	1.6019	10.826	17,462	2,359	980,727	373.370	3.259
4j	617.156	332.831	283.424	1.5950	10.501	14,426	1,817	674,005	342.399	5.061
4k	640.929	346.431	295.399	1.6151	10.808	16,192	2,300	957,097	374.398	4.058
4l	594.132	518.705	270.606	1.5752	10.190	13,990	1,871	674,005	344.372	4.184
4m	626.359	337.859	288.675	1.5995	10.826	16,700	2,245	934,720	373.370	3.259
4n	591.876	317.924	270.660	1.5711	10.190	13,774	1,833	660,855	344.372	4.184
Std. 1*	487.133	245.631	192.36	1.5242	7.183	6,809	981	272,224	275.222	–
Std. 2*	570.267	318.963	282.947	1.5303	9.792	13,519	2,039	748,946	404.350	2.419
Std. 3*	537.301	278.198	221.39	1.5699	8.2029	8,433	1,195	399,912	301.303	1.801
Std. 4*	580.224	331.272	320.889	1.46146	10.691	15,459	2,353	1,046,550	437.46	–

SAS Connolly solvent accessible surface area, *MS* Connolly molecular surface area, *CSEV* Connolly solvent excluded volume, *MR* molar refractivity *MTI* molecular topological index, *WI* Wiener index, *BI* Balaben index, *MW* molecular weight

to define various types of biological activity, due to its relationship with membrane permeability. In order to identify substituent effect on antimicrobial activity, quantitative structure–activity relationship (QSAR) studies of title compounds were performed. Biological activity data i.e., minimum inhibitory concentration (MIC) was converted to the logarithmic value (pMIC) in μg/ml as listed in Tables 4 and 5. The compounds were analyzed by physicochemical-based QSAR (Hansch) approach using different physicochemical parameters as independent and pMIC values as dependent variables. These QSAR descriptors of substituted hydrazides were calculated using the Sigmastat package 3.5. The 14 test compounds are listed in Tables 4 and 5 along with their pMIC log *P* and ovality values considered for QSAR development.

The basic structure of test compounds is:



A number of QSAR equations were developed by taking into consideration of various parameters and relating them to MIC values of the test compounds against various bacterial and fungal strains. Among *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*, the best QSAR equations were obtained by relating pMICsa, pMICec, and pMICca to log *P* values by polynomial regression analysis (Table 4). Log *P* is a measure of hydrophobicity, which is important for the penetration, distribution as well as for the interaction of the drug with receptors. Therefore, it can be suggested that lipophilic

Table 3 % Age similarities of target compounds with standard drugs

Compounds	Nifuroxazide $(1 - R)$ 100	Tosufloxacin tosylate $(1 - R)$ 100	Pyridoxal salicyloyl hydrazone $(1 - R)$ 100	Cefexime $(1 - R)$ 100
4a	85.79	90.86	72.58	82.24
4b	61.36	89.05	71.92	87.32
4c	57.67	92.94	77.07	86.91
4d	96.86	77.23	55.63	83.52
4e	42.49	74.70	52.55	88.48
4f	60.63	92.47	76.56	87.38
4g	87.46	71.23	48.84	92.02
4h	81.96	62.05	37.26	96.06
4i	78.99	85.45	68.53	98.38
4j	83.88	62.05	38.09	94.88
4k	78.62	74.40	54.27	98.68
4l	85.10	75.65	54.81	93.72
4m	79.68	85.92	69.16	97.77
4n	85.25	75.77	54.97	93.57

Table 4 Comparison of logarithmic values (pMIC) of minimum inhibitory concentration (MIC) to log *P*

Compounds	*R*′	Ar	pMICsa	PMICec	pMICca	Log *P*
4a	4-Cl	2-OHC_6H_4	1.255	1.301	1.740	3.532
4b	4-Cl	3-OCH_3, 4-OHC_6H_3	1.255	1.531	1.924	3.406
4c	4-Cl	4-NO_2·C_6H_4	1.301	1.531	1.301	3.133
4d	4-Cl	4-CH_3·C_6H_4	1.477	2.264	1.301	4.409
4e	4-Cl	2-$OHC_{10}H_6$	1.477	1.924	1.531	4.53
4f	4-Cl	3-$NO_2C_6H_4$	1.301	1.301	1.531	3.133
4g	2-COC_6H_5	–C_6H_5	1.477	1.924	2.000	4.573
4h	2-COC_6H_5	4-Cl·C_6H_4	1.531	1.924	2.301	5.132
4i	2-COC_6H_5	4-NO_2·C_6H_4	1.531	1.397	1.924	3.259
4j	2-COC_6H_5	4-CH_3·C_6H_4	1.531	2.225	1.698	5.061
4k	2-COC_6H_5	3-OCH_3, 4-OHC_6H_3	1.531	1.698	1.301	4.058
4l	2-COC_6H_5	4-OHC_6H_4	1.531	1.903	2.000	4.184
4m	2-COC_6H_5	2-NO_2·C_6H_4	1.255	1.301	1.397	3.259
4n	2-COC_6H_5	2-OHC_6H_4	1.301	1.531	2.000	4.184

Table 5 Comparison of logarithmic values (pMIC) of minimum inhibitory concentration (MIC) to ovality (*X*)

Compounds	*R*′	Ar	$pMIC_{se}$	$pMIC_{pa}$	$pMIC_{an}$	Ovality (*X*)
4a	4-Cl	2-OHC_6H_4	1.53	1.09	1.00	1.5148
4b	4-Cl	3-OCH_3, 4-OHC_6H_3	2.00	1.40	1.69	1.5591
4c	4-Cl	4-NO_2·C_6H_4	0.00	1.30	0.81	1.5372
4d	4-Cl	4-CH_3·C_6H_4	2.30	0.81	0.81	1.5394
4e	4-Cl	2-$OHC_{10}H_6$	2.00	1.40	2.00	1.5255
4f	4-Cl	3-$NO_2C_6H_4$	1.69	1.40	0.81	1.5616
4g	2-COC_6H_5	–C_6H_5	1.69	0.81	0.81	1.5666
4h	2-COC_6H_5	4-Cl·C_6H_4	1.69	1.09	1.30	1.5844
4i	2-COC_6H_5	4-NO_2·C_6H_4	1.47	1.30	1.40	1.6019
4j	2-COC_6H_5	4-CH_3·C_6H_4	2.30	1.40	0.81	1.5950
4k	2-COC_6H_5	3-OCH_3, 4-OHC_6H_3	2.30	1.69	1.40	1.6151
4l	2-COC_6H_5	4-OHC_6H_4	1.92	1.40	1.92	1.5752
4m	2-COC_6H_5	2-NO_2·C_6H_4	1.47	1.40	1.09	1.5995
4n	2-COC_6H_5	2-OHC_6H_4	1.53	1.09	1.53	1.5711

properties have to be checked for designing of potent antibacterial agents as these are vital factors for its activity.

QSAR model for antibacterial activity against *Staphylococcus aureus*

$$\text{pMICsa} = -19.98\,(\text{Log}\,P)^2 + 59.53\,\text{Log}\,P - 40.53 \quad \text{(i)}$$

$n = 14$, $r = 0.682$, $r^2 = 0.466$, $s = 0.122$, $s^2 = 0.015$, $F = 10.064$

Here and thereafter, r = coefficient of correlation, r^2 = coefficient of determination, s^2 = sum of squares of deviations of sample values (variance), s = standard deviation i.e., square root of variance, F = Fischer statistics, n = number of test compounds

On exclusion of compound GS_9, Eq. (ii) was obtained with better statistical validation:

$$\text{pMICsa} = -6.030\,(\text{Log}\,P)^2 + 21.78\ \text{Log}\ P - 14.58 \quad \text{(ii)}$$

$n = 13$, $r = 0.826$, $q^2 = 0.683$, $s = 0.120$, $s^2 = 0.014$, $F = 24.372$, here and thereafter, q^2 cross validated r^2 obtained by leave one out (LOO) technique.

This equation showed an improved relationship between MIC values and log *P* e.g., value of *r* was 0.826 (vs 0.682 in Eq. i) and *s* value was 0.120 (vs 0.122 in Eq. i).

The negative coefficient of Log *P* in the Eq. (i) indicates that there is a negative correlation between the antibacterial activity of substituted hydrazides and Log *P*. This is evidenced by the antibacterial activity data of substituted hydrazides and their Log *P* values.

QSAR model for antibacterial activity against *Escherichia coli*

$$\text{pMICec} = -1.126\,(\text{Log}\,P)^2 + 5.730\,\text{Log}\,P - 2.389 \quad \text{(iii)}$$

$n = 14,\ r = 0.865,\ r^2 = 0.758,\ s = 0.121,\ s^2 = 0.014,\ F = 31.918$

On exclusion of compound GS_4, Eq. (iv) was obtained with better statistical validation:

$$\text{pMICec} = -0.149\,(\text{Log}\,P)^2 + 1.626\,\text{Log}\,P - 0.846 \quad \text{(iv)}$$

$n = 13,\ r = 0.894,\ q^2 = 0.801,\ s = 0.118,\ s^2 = 0.013,\ F = 44.228$

This equation showed an improved relationship between MIC values and log P e.g., value of r was 0.894 (vs 0.865 in Eq. iii) and s value was 0.118 (vs 0.121 in Eq. iii).

QSAR model for antibacterial activity against *Candida albicans*

$$\text{pMICca} = -2.161\,(\text{Log}\,P)^2 + 6.673\,\text{Log}\,P - 8.871 \quad \text{(v)}$$

$n = 14,\ r = 0.462,\ r^2 = 0.214,\ s = 0.323,\ s^2 = 0.104,\ F = 0.902$

On exclusion of compound GS_{10}, Eq. (vi) was obtained with better statistical validation:

$$\text{pMICca} = -6.030\,(\text{Log}\ P)^2 + 21.78\ \text{Log}\ P\ - 14.58 \quad \text{(vi)}$$

$n = 13,\ r = 0.637,\ q^2 = 0.407,\ s = 0.303,\ s^2 = 0.091,\ F = 0.483$

This equation showed an improved relationship between MIC values and log P e.g., value of r was 0.637 (vs 0.462 in Eq. v) and s value was 0.303 (vs 0.323 in Eq. v).

Similarly, a number of QSAR equations were developed by taking into consideration of various parameters and relating them to MIC values of the test compounds against *Staphylococcus epidermidis*, *Pseudomonas aeruginosa,* and *Aspergillus niger*. Among these, the best QSAR equations were obtained by relating pMICse, pMICpa and pMICan to ovality (X) values by polynomial regression analysis (Table 5).

QSAR model for antibacterial activity against *Staphylococcus epidermidis*

$$\text{pMICse} = 0.179\,(X)^2 - 0.678(X) + 2.193 \quad \text{(vii)}$$

$n = 14,\ r = 0.602,\ r^2 = 0.363,\ s = 0.210,\ s^2 = 0.044,\ F = 5.792$

On exclusion of compound GS_4, Eq. (viii) was obtained with better statistical validation:

$$\text{pMICec} = 0.247(X)^2 - 0.915(X) + 2.395 \quad \text{(viii)}$$

$n = 13,\ r = 0.685,\ q^2 = 0.456,\ s = 0.193,\ s^2 = 0.037,\ F = 1.294$

This equation showed an improved relationship between MIC values and log P e.g., value of r was 0.685 (vs 0.602 in Eq. vii), and s value was 0.193 (vs 0.210 in Eq. vii).

QSAR model for antibacterial activity against *Pseudomonas aeruginosa*

$$\text{pMICca} = 0.191(X)^2 - 0.550\,(X) + 1.197 \quad \text{(ix)}$$

$n = 14,\ r = 0.634,\ r^2 = 0.403,\ s = 0.262,\ s^2 = 0.068,\ F = 0.0208$

On exclusion of compound GS_{10}, Eq. (ix) was obtained with better statistical validation:

$$\text{pMICca} = 0.210(X)^2 - 0.613\,(X) + 1.146 \quad \text{(x)}$$

$n = 13,\ r = 0.575,\ q^2 = 0.758,\ s = 0.259,\ s^2 = 0.067,\ F = 0.00617$

This equation showed an improved relationship between MIC values and log P e.g., value of r was 0.758 (vs 0.634 in Eq. ix), and s value was 0.259 (vs 0.262 in Eq. ix).

All the considered regression models must pass a test based on the value of Fisher statistics of all their regression coefficients. Good values of the F-test indicate that the model is statistically significant, but in Eq. (x) as the value of F-ratio is low indicates this model is less significant.

QSAR model for antibacterial activity against *Aspergillus niger*

$$\text{pMICca} = 0.111(X)^2 - 0.216\,(X) + 1.657 \quad \text{(xi)}$$

$n = 14,\ r = 0.489,\ r^2 = 0.240,\ s = 0.254,\ s^2 = 0.064,\ F = 2.565$

On exclusion of compound GS_5, Eq. (xi) was obtained with better statistical validation:

$$\text{pMICca} = 0.093(X)^2 - 0.168\,(X)\ -\ 1.620 \quad \text{(xii)}$$

$n = 13,\ r = 0.586,\ q^2 = 0.344,\ s = 0.247,\ s^2 = 0.061,\ F = 2.520$

This equation showed an improved relationship between MIC values and log P e.g., value of r was 0.637 (vs 0.462 in Eq. xi), and s value was 0.303 (vs 0.323 in Eq. xi).

The statistical data so obtained demonstrates the role of the parameter ovality (X) in antimicrobial activity. The parameter ovality is a physicochemical parameter and it involves a combined effect of size and surface area of the substituents. It characterizes deformation of molecular electrons distribution. The equations also showed the direct relationship between ovality and pMIC i.e., an increase in ovality (X) enhances the antimicrobial activity of compounds.

Pharmacological evaluation

*In vitro anti-inflammatory activity of synthesized compounds (**4a–n**)*

The synthesized compounds were screened for anti-inflammatory activity using inhibition of albumin denaturation technique which was studied according to Muzushima and Kabayashi with slight modification (Panneerselvam *et al.*, 2010; Rao *et al.*, 2005; Chanda and Dave 2009). The standard

drug and test compounds were dissolved in minimum amount of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solutions was less than 2.5 %. Test solution (1 ml) containing different concentrations of drug was mixed with 1 ml of 1 mM albumin solution in phosphate buffer and incubated at 27 ± 1 °C in BOD incubator for 15 min.

Denaturation was induced by keeping the reaction mixture at 60 ± 10 °C in water bath for 10 min. After cooling, the turbidity was measured at 660 nm by UV–Visible spectrophotometer.

Percentage of inhibition of denaturation was calculated from control (without drug) using the following formula:

$$\%\,\text{age inhibition} = (A_0 - A_1/A_0) \times 100$$

where, A_0 is the absorbance of control and A_1 is the absorbance of test. Diclofenac was used as standard.

Each experiment was done in triplicate and average was taken. Also it was found that **4a**, **4c**, **4d**, and **4e** compounds lead to considerable inhibition of denaturation. Out of these, **4e** was most active. The IC_{50} values of the synthesized compounds **4a**–**n** were calculated as given in Table 6. The activity of all the compounds was compared with diclofenac (standard). The compounds containing nitro, alkyl, and β-naphthyl substituent showed maximum activity.

*In vitro antioxidant activity of synthesized compounds (**4a**–**n**)*

Hydrogen peroxide radical scavenging (H_2O_2) assay In the body, H_2O_2 led to production of hydroxyl radicals (OḢ) which cause lipid peroxidation and DNA damage. The ability of synthesized compounds to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.*, (1989) (Panneerselvam *et al.*, 2010). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using UV–Visible spectrophotometer.

The sample solution (20–60 µg/ml) in distilled water was added to hydrogen peroxide and absorbance at 230 nm was determined after 10 min against a blank solution that contained drug solution in phosphate buffer without hydrogen peroxide.

The IC_{50} values of the synthesized compounds **4a**–**n** were calculated as given in Table 6. These experiments suggest that the nitro, alkyl, and hydroxyl substituent were one of the key groups to enhance the antioxidant activity mainly due to its redox property through one electron transfer mechanism.

Total antioxidant activity The antioxidant activity of the compound **4a**–**n** was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.*, (1999).The assay was based on the reduction of Mo (VI)–Mo (V) by the test solution and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. Compounds were dissolved in $CHCl_3$:ethanol (2:1) and different concentrations of compounds (250–1,000 µg/ml) were combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95 °C for 60 min.

Then the absorbance of the solution was measured at 695 nm using double beam UV–Visible spectrophotometer against blank (with 3 ml of reagent solution without using test

Table 6 IC_{50} (µg/ml) values of anti-inflammatory activity the different synthesized hydrazones (**4a**–**n**)

S. no.	Compounds	IC_{50} (µg/ml)
1	**4a**	95.00
2	**4b**	160.00
3	**4c**	85.00
4	**4d**	86.00
5	**4e**	80.00
6	**4f**	124.00
7	**4g**	105.00
8	**4h**	54.00
19	**4i**	17.00
10	**4j**	12.00
11	**4k**	10.00
12	**4l**	20.00
13	**4m**	75.00
14	**4n**	35
15	Diclofenac (standard)	71.00

Table 7 IC_{50} (µg/ml) values of antioxidant activity of the different synthesized hydrazones

Compounds	Total antioxidant activity IC_{50} (µg/ml)	H_2O_2 scavenging activity IC_{50} (µg/ml)
4a	50.00	34.00
4b	32.00	17.00
4c	45.00	01.80
4d	42.00	04.30
4e	120.00	69.00
4f	30.00	22.00
4g	49.00	152.00
4h	20.00	109.00
4i	18.00	125.00
4j	5.00	84.00
4k	70.00	79.00
4l	100.00	91.00
4m	42.00	101.00
4n	15.00	132.00
4a	55.00	46.00
4b	41.00	00.50

Table 8 Minimum inhibitory concentration (MIC) in μg/ml of synthesized compounds

Compounds	Minimum inhibitory concentration (MIC) in μg/ml					
	EC	PA	SE	SA	CA	AN
4a	20	12.5	34	18	55	10
4b	34	25	100	18	84	50
4c	34	20	100	20	20	10
4d	184	6.5	200	30	20	10
4e	84	25	100	30	34	100
4f	20	25	50	20	34	10
4g	84	6.5	50	30	100	10
4h	84	12.5	50	34	200	20
4i	25	20	30	34	84	25
4j	168	25	200	34	50	10
4k	50	50	200	34	20	25
4l	80	25	84	34	100	84
4m	20	25	30	18	25	10
4n	34	12.5	34	20	100	34
Control	–	–	–	–	–	–
Amoxycillin	12.5	200	100	12.5	–	–
Fluconazole	–	–	–	–	12.5	400
Cefixime	50	400	400	50	–	–

EC Escherichia coli; *PA Pseudomonas aeruginosa*; *SE Staphylococcus epidermidis*; *SA Staphylococcus aureus*; *CA Candida albicans*; *AN Aspergillus niger*

solution) after cooling to room temperature. The antioxidant activity was compared with tannic acid used as standard.

The IC_{50} values of the synthesized compounds **4a**–**n** were calculated and given in Table 7. These experiments suggested that the alkyl, hydroxyl, and 3-nitro substituent were one of the key groups to enhance greatly the antioxidant activity of substituted hydrazones mainly due to its redox property through the one electron transfer mechanism that may attribute the antioxidant properties. Compound **4b** and **4f** showed maximum antioxidant activity.

Antimicrobial activity

The antibacterial activities of the compounds (**4a**–**n**) were tested against Gram-positive bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis* and Gram-negative bacteria *Escherchia coli*, *Pseudomonas aeruginosa*. The antifungal activities of the compounds (**4a**–**n**) were tested against *Candida albicans* and *Aspergillus niger*. The results of in vitro antimicrobial activities of the hydrazones are listed in Table 8. Minimum inhibitory concentration (MIC) of target compounds was measured against amoxycillin, fluconazole, and cefixime (reference drugs) as described in Table 8. All the compounds showed good antimicrobial activity against the tested bacteria in comparison to cefixime as positive control.

Result and discussion

Chemistry

The target compounds (**4a**–**n**) were prepared as outlined in Schemes 1 and 2. The key intermediate was obtained by Fischer esterification of 4-chlorobenzoic acid with the absolute ethanol in the presence of concentrated sulfuric acid, and hydrazide was obtained by refluxing ester with hydrazine hydrate. Further hydrazone was obtained by refluxing hydrazide with substituted aromatic aldehyde in the presence of glacial acetic acid. Finally, the new target compounds **4a**–**n** were obtained, in good yields (71–99 %), by condensing hydrazides with the corresponding appropriate aldehydes in absolute ethanol. The purity and authenticity of compounds were established by chromatographic and spectroscopic means. The structures assigned to **4a**–**n** were supported by IR spectra showing absorption bands at 3,427–3,280 cm^{-1} and 3,402–3,200 cm^{-1} due to O–H and N–H stretching, respectively. Band at 1,676–1,620 cm^{-1} indicated C=O stretch. Stretching vibrations due to C=N and C=C (aromatic) were observed at 1,680–1,409 cm^{-1} and 1,620–1,500 cm^{-1}, respectively. Band at 1,529.55–1,456 cm^{-1} was appeared due to asymmetric N=O stretch. Bands at 1,296–1,276 cm^{-1} indicated C–O–C stretch. Bands at 854–785 cm^{-1} indicated out of plane aromatic stretch. A characteristic strong band at 726–611 cm^{-1} was due to the aliphatic C–Cl stretching vibrations. The proton NMR of these compounds revealed the presence of singlet 12.50–10.70 ppm for –NH–N=. The –N=CH– proton was observed as broad singlet 8.56–6.8 ppm. A singlet due to proton of –OH appeared at δ 5.80. A 3 proton singlet at δ 3.83 appeared corresponding to methoxy protons. A 3 proton singlet at δ 2.40 appeared corresponded to the protons of methyl group. All the other aliphatic and aromatic protons were observed within the expected regions.

Biological activity

Antimicrobial activity of synthesized hydrazone analogs was carried out by agar diffusion method and the average radius of zone of inhibition (in mm) and MIC (in μg/ml) was recorded. All the synthesized derivatives were screened for antimicrobial activity and compared with the standard drugs amoxycillin, cefixime (for antibacterial activity), and fluconazole (for antifungal activity).

Antifungal activity of all the compounds was good but nothing was found to be more active compared to fluconazole (reference drug). While studying MIC against bacterial strains **4c**, **4f**, **4i**, **4k**, and **4n** were most active among

all the target compounds. The compound **4d** was found to be more active against *Staphylococcus aureus* than *Staphylococcus epidermidis.* All target compounds were found to be active against *Staphylococcus aureus* and *Pseudomonas aeruginosa* where as some of them were found to be less active against *Escherichia coli* and *Staphylococcus epidermidis.* The compound **4m** was found to be least active in *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*, but exhibited good activity against the remaining bacterial and fungal strains.

While studying the antifungal activity **4c**, **4d**, **4e**, **4f,** and **4k** were found to be moderately active. The **4i** was found to be highly active against all the target compounds. Among all the target compounds **4e**, **4g**, and **4i** were least active. It was found that target compounds were more active against *Aspergillus niger* than *Candida albicans.* Activity against *Aspergillus niger* was even more than standard drug. It was found that hydrazone derivatives with 2-nitro and 4-nitrophenyl substitution showed good antibacterial property. The presence of methyl and methoxy group on phenyl ring was found to have no impact in improving the antibacterial activity.

All the target compounds were also studied for their antioxidant activity using two in vitro antioxidant activity models; total antioxidant activity and hydrogen peroxide scavenging activity.

While studying total antioxidant activity, all target compounds were found to have good antioxidant activity.

Among all the target compounds **4a**, **4b**, **4c**, **4d**, **4f**, **4g**, **4h**, **4i**, **4j**, **4k**, **4i**, **4m**, and **4n** were found to be active than ascorbic acid (standard). Out of all the active compounds **4b**, **4f**, **4g**, **4h**, **4i**, **4j**, and **4n** were even active than second reference drug tannic acid. While studying the hydrogen peroxide scavenging activity, we found that **4a**, **4b**, **4c**, **4d**, and **4f** were more active than ascorbic acid (standard). None of them was active than second standard tannic acid. These studies suggested that the nitro, alkyl, and hydroxyl substituent were one of the key groups to enhance the antioxidant activity mainly due to its redox property through the one electron transfer mechanism.

The compounds were screened for their in vitro anti-inflammatory activity, and this was done by means of studying albumin denaturation studies. It was found that **4a**, **4c**, **4d**, and **4e** compounds lead to considerable inhibition of denaturation. IC_{50} of the compounds were also calculated as showed in Table 4. Compounds **4c**, **4d**, and **4e** demonstrated better activity which was very close to that of diclofenac (standard). Out of these, **4e** showed maximum activity.

Structure similarity studies

Assessment of structural similarities of target compounds with standard drugs showed that all compounds have good percentage similarity except **4e**. All the synthesized compounds showed good structural similarity with cefixime and tosufloxacin tosylate as compared to other standard drugs. It was found that synthesized compounds have least similarity to pyridoxal salicyloyl hydrazone. Least structural similarity of target compounds to pyridoxal salicyloyl hydrazone was due to the absence of pyridine moiety in target compounds. Further compounds **4g** and **4n** have very low percentage similarity due to the presence of *o*-benzoyl on aromatic ring.

Quantitative structure activity relationship

From the analysis of structures and the activity, it was displayed that some structure–activity relationships can be extracted in target compounds. The structural requirements for antibacterial and antifungal activity are different for substituted hydrazides. This was evidenced by the fact that the some active antibacterial compounds **4g**, **4i**, and **4n** compounds showed least antifungal activity and compounds **4d** and **4j** being the most active antifungal compounds have shown least antibacterial activity. The presence of electron-withdrawing groups ($–NO_2$, –Cl, –Br, –OH) on aromatic ring improved the antimicrobial activity of compounds.

The analysis of structures of most active antibacterial compounds may be concluded that among the electron-withdrawing groups ($–NO_2$, –Cl, –Br), the halo groups on the aromatic ring are necessary for the antimicrobial activity. Further, it may be possible that the introduction of electron-withdrawing halo group on the second aromatic ring may further improve the antimicrobial activity of these compounds. This indicated that the introduction of –Cl and $–NO_2$ group may improve antibacterial and antifungal activity respectively.

Conclusion

Assessment of structural similarities of target compounds with standard drugs showed that all compounds have good % age similarity except **4e**. All the synthesized compounds showed good structural similarity with cefixime and tosufloxacin tosylate as compared to other standard drugs. It was found that synthesized compounds have least similarity to pyridoxal salicyloyl hydrazone. Least structural similarity of target compounds to pyridoxal salicyloyl hydrazone was due to the absence of pyridine moiety in target compounds. Further, compounds **4g** and **4n** have very low percentage similarity due to the presence of *o*-benzoyl on aromatic ring.

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The results provided basic information to establish that the nature of the substituent on aromatic ring was very essential for various types of activities for example in present study the presence of 4-methyl (**4d**), 3-nitro (**4f**), and 4-nitro (**4c**) leads to good antioxidant activity whereas the presence of 4-hydroxy (**4a**) do not contribute much for antioxidant activity. These studies suggested that the nitro, alkyl, and hydroxyl substituent were one of the key groups to enhance the antioxidant activity mainly due to its redox property through the one electron transfer mechanism.

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