Full Paper

Synthesis, Antihypertensive Activity, and 3D-QSAR Studies of Some New *p*-Hydroxybenzohydrazide Derivatives

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p-Hydroxybenzohydrazide **2** on treatment with aromatic/aliphatic aldehyde followed by cyclization with carbon disulphide afforded compounds **4a–4n**. Also, compound **2** by treatment of substituted isothiocyanate followed by the treatment of chloroacetic acid yields the corresponding compounds **6a–6i**. All the test compounds were assayed for antihypertensive activity by non-invasive blood pressure measurement and invasive blood pressure measurement methods. The test compounds showed significant antihypertensive activity. The intact compounds were subjected to 3D-QSAR studies. The 3D-QSAR analysis was carried out by PHASE program and a statistically reliable model with good predictive power ($r^2 = 0.98$, $q^2 = 0.74$) was achieved. The 3D-QSAR plots illustrated insights into the structure-activity relationship of these compounds which may aid in the design of potent *p*-hydroxybenzohydrazide derivatives as antihypertensive agents.

Keywords: Antihypertensive activity / PHASE program / 3D-QSAR / Synthesis

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Introduction

High blood pressure (BP) is one of the most potent risk factors for the first and recurrent stroke [1]. One third of the world population is affected with cardiovascular diseases and the major part of it stems from hypertension [2–4]. Hypertension is recognized as a major risk factor in a human patient with cerebral hemorrhage and heart and renal disease; therefore, diagnosis of hypertension is carried out by measuring blood pressure on a regular basis [5–7]. *p*-Hydroxybenzohydrazide analogs seem to be suitable parent compounds from which a variety of biological activities are reported such as antitumor [8, 9], anti-anginal [10], antitubercular [11, 12], antihypertensive [13, 14], MAO (monoamine oxidase) enzyme inhibitor [15], antibacterial [16], etc. Discovering three-dimensional pharmacophores which can explain the activity of a series of ligands is one of the most significant contributions of computational chemistry to drug discovery [16]. Quantitative drug design embraces two major activities, the quantitative description of the structural differences among a series of chemical compounds of biological interest, and the formulation of ''QSAR'' (quantitative structure–activity relationship) useful in the design of new and better therapeutic agents [17]. A QSAR is a mathematical relationship between a biological activity of a molecular system and its geometric and chemical characteristics. QSAR attempts to find a consistent relationship between biological activity and molecular properties, so that these ''rules'' can be used to evaluate the activity of new compounds: 3D models are more easily interpretable than two-dimensional descriptors or fingerprint-based QSAR models making it easier to suggest new compounds for synthesis.

The chemistry of *p*-hydroxybenzohydrazide is of great interest. This moiety has an immense importance for its activity access against many diseases. In recent years, interest has also been focused on thiadiazole derivatives and thiazolidine derivatives of benzohydrazide [18–21].

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Abbreviations: blood pressure (BP); invasive blood pressure measurement (IBP); non-invasive blood pressure measurement (NIBP); partial least square (PLS); Quantitative Structure-Activity Relationship (QSAR).

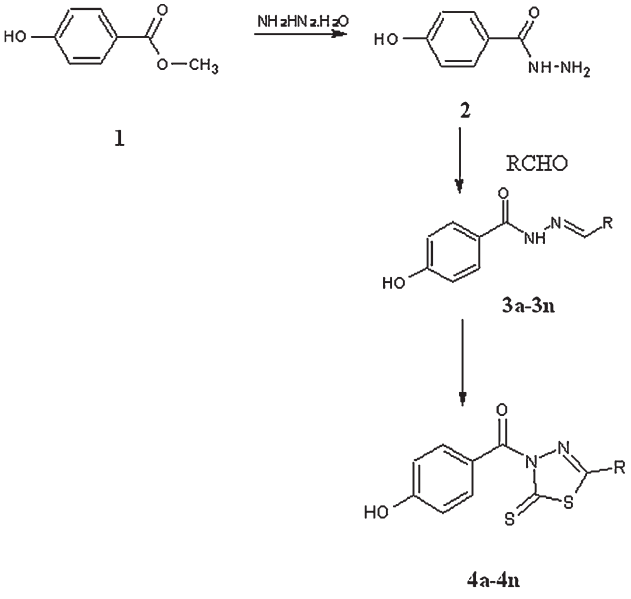
Results

Synthesis

Some novel (4-hydroxyphenyl)-[(5-substituted alkyl/aryl)-2-thioxo-1,3,4-thiadiazol-3-yl]methanones and *N*′-[(3-substituted alkyl/

aryl)-4-oxo-1,3-thiazolidin-2-ylidene]-4-hydroxybenzohydrazide were synthesized with the aim of obtaining new agents which might have more or a similar antihypertensive activity than known ones. Thus, in the present investigation, 14 different derivatives of (4-hydroxyphenyl)-[5-substituted alkyl/aryl)-2-thioxo-1,3,4-thiadiazol-3-yl]methanone and nine derivatives of *N*'-[(3-substituted alkyl/aryl)-4-oxo-1,3-thiazolidin-2-ylidene]-4-hydroxybenzohydrazide were synthesized and were evaluated for their antihypertensive activity. The synthesis of the intermediate and target compounds was performed by the reactions illustrated in Schemes 1 and 2.

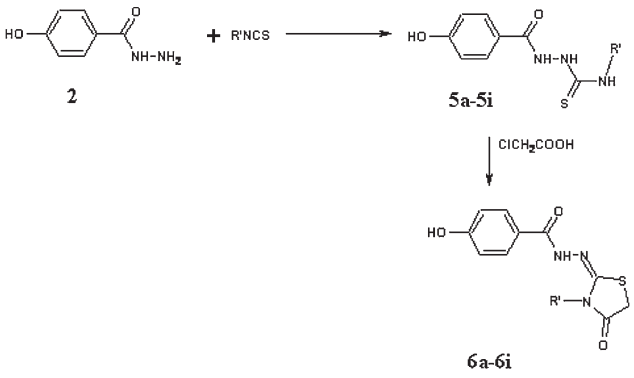
Compound **2**, 4-hydroxybenzohydrazide, was synthesized by amination of compound **1** by hydrazine hydrate (80%; Scheme 1). The physical and elemental analysis data confirmed the formation of the compound **2**. To a solution of compound **2** in ethanol various aliphatic/aromatic aldehydes were added. The mixture was refluxed and excess solvent was distilled off to afforded *N*'-[(substituted alkyl/aryl)-methylidene]-4-hydroxybenzohydrazide **3a–3n**. To a solution of compounds **3a–3n** in ethanol, carbon disulphide in alc. KOH was added to obtained cyclized (4-hydroxyphenyl)-[(5-substituted-alkyl/aryl)-2-thioxo-1,3,4-thiadiazol-3-yl]-methanones **4a–4n**. The formation of various imines (Schiff's bases) **3a–3n** takes place by the elimination of water from the compounds. The excess of water was removed. Further, the nitrogen in the side chain of *N*'-[(substituted alkyl/aryl) methylidene]-4-hydroxybenzohydrazide, with its lone pair of electrons, attacks the carbon atom of carbon disulphide to give the intermediate, which – on intermolecular rearrangement – afford the cyclized products **4a–4n**.



1 **2** **3a-3n** **4a-4n**

Compound		R
3a	**4a**	Isopropyl
3b	**4b**	*n*-Butyl
3c	**4c**	Phenyl
3d	**4d**	4-Nitrophenyl
3e	**4e**	4-Chlorophenyl
3f	**4f**	4-Fluorophenyl
3g	**4g**	2,6-Dichlorophenyl
3h	**4h**	4-Methylphenyl
3i	**4i**	4-Hydroxyphenyl
3j	**4j**	4-Methoxyphenyl
3k	**4k**	3-Hydroxy-4-methoxyphenyl
3l	**4l**	3,4-Dimethoxyphenyl
3m	**4m**	*N,N*-Dimethylaminophenyl
3n	**4n**	Furyl

Scheme 1. Synthesis of compounds **3a–n** and **4a–n**.



2 **5a-5i** **6a-6i**

Compound		R
5a	**6a**	Isoproyl
5b	**6b**	*n*-Butyl
5c	**6c**	Phenyl
5d	**6d**	4-Nitrophenyl
5e	**6e**	4-Fluorophenyl
5f	**6f**	2,4-Dichlorophenyl
5g	**6g**	2,6-Difluorophenyl
5h	**6h**	2,6-Dimethylphenyl
5i	**6i**	2,4-Dimethoxyphenyl

Scheme 2. Synthesis of compounds **5a–i** and **6a–i**.

The IR spectra of compounds **3a–3n** showed absorption bands at 1500–1569 cm^{-1} (C=N stretching) and 1658–1684 cm^{-1} (C=O stretching) due to the conversion of amine to imine. Compound **3a** showed the presence of characteristic absorption peaks at 2986 and 2990 cm^{-1} ($CH(CH_3)_2$ stretching); compound **3b** showed absorption peaks at 2981, 2986, and 2990 cm^{-1} ($CH_2CH_2CH_2CH_3$ stretching);

compound **3d** showed an absorption peak at 1498 cm^{-1} (C-NO_2 stretching); compounds **3e** and **3g** have absorption peaks at 1080–1092 cm^{-1} (Ar-Cl stretching), compound **3f** at 1035 cm^{-1} (Ar-F stretching). Absorption peaks of compound **3h** are at 3010–3030 cm^{-1} (Ar-CH stretching); compound **3i** showed an absorption band at 3537 cm^{-1} (Ar-OH stretching) and compounds **3j** and **3l** at 1050–1150 cm^{-1} (O-CH_3 stretching); finally, compound **3n** showed an absorption band at 1600 cm^{-1} (furan), The compounds **4a–4n** showed absorption bands at about 1189–1290 cm^{-1} and 752–760 cm^{-1} (C-S-C stretching) due to presence of the thioxo-thiadiazole ring. The disappearance of the peak at 1500–1569 cm^{-1} (C=N) confirms the assigned structure.

In ^{1}H-NMR spectra of compounds **3a–3n**, the imine proton appeared as a multiplet at $\delta = 2.1$–2.3 ppm integrating for one proton. The signal due to an aromatic proton appeared as multiplet in the range of δ values 7.2–8.1 ppm integrating for four protons. The cyclized compounds **4a–4n** showed the absence of a signal at $\delta = 2.1$–2.3 for imine. The compounds showed a characteristic peak at $\delta = 5.33$ (s, 1H, Ar-OH). Compound **4a** showed a peak at $\delta = 1.31$–1.5 ppm (d, CH-$(CH_3)_2$), while **4b** showed peaks at $\delta = \delta$ 0.95 (t, 3H, butyl CH_3), 1.28 (m, 2H, butyl CH_2), 1.51 (m, 2H, butyl CH_2), 3.53 ppm (m, 2H, butyl CH_2). Compound **4h** had a peak at $\delta = 2.2$ ppm (Ar-CH_3) and compounds **4j** and **4l** showed singlets at $\delta = 1.2$–1.31 ppm (Ar-OCH_3); in compound **4m**, a singlet is seen at $\delta = 4.2$–4.4 ppm ($N(CH_3)_2$), whereas compound **4n** showed a triplet at $\delta = 6.85$–8.5 ppm (furan). Other peaks, in addition, confirm the structures assigned.

In the EI-MS spectra, the molecular ion peaks [M^+], which appeared at different intensities, confirmed the molecular weight of compounds **3a–3n**. Molecular ion peaks are the base peaks for the compounds. The appearance of an isotopic peak [M + 2] along with the molecular ion peak confirmed the presence of sulphur atoms in compounds **4a–4n**.

Addition of substituted isothiocyanates to the solution of compound **2** in ethanol afforded the corresponding 2-[(4-hydroxyphenyl) carbonyl]-*N*-substituted hydrazine carbothioamide **5a–5i**. In this reaction, compound **2** acts as a nucleophile, having a lone pair of electrons on the nitrogen. The reaction of **5a–5i** with chloroacetic acid in boiling ethanol containing fused sodium acetate undergoes a nucleophilic addition–elimination reaction affording the corresponding *N*'-[(3-substituted alkyl/aryl)-4-oxo-1,3-thiazolidin)-2-ylidene]-4-hydroxybenzohydrazides **6a–6i**.

The IR spectra of compounds **5a–5i** showed NH and CS stretching bands at 3215–3230 and 1309–1348 cm^{-1}, respectively. Compound **5a** showed the presence of characteristic absorption peaks at 2986 and 2990 cm^{-1} ($CH(CH_3)_2$stretching); **5b** showed absorption peaks at 2984, 2989, and 2991 cm^{-1} ($CH_2CH_2CH_2CH_3$ stretching), and **5d** showed a band at 1555 cm^{-1} (C-NO_2 stretching). Compounds **5e** and **5g** showed absorption peaks at 1045 cm^{-1} (Ar-F stretching), compound **5f** has absorption peaks at 700–750 cm^{-1} due to C-Cl stretching, **5h** showed a peak at 3010–3030 cm^{-1} (Ar-CH stretching), and compound **5i** showed a peak at 1050–1150 cm^{-1} (O-CH_3 stretching). The IR spectra of the cyclized compounds **6a–6i** revealed the disappearance of the NH band at 3215–3230 cm^{-1}.

The ^{1}H-NMR of compounds **5a–5i** showed a downfield signal at $\delta = 10.80$–11.81 ppm attributed to 3-substitued NH. The signal due to aromatic protons appeared as a multiplet in the range of $\delta = 7.2$–8.1 ppm integrating for nine protons. The cyclized compounds **6a–6i** lacked the NH signals and showed a new singlet signal at $\delta = 3.01$–3.98 ppm attributable to the thiazoline ring. Compounds **5a** and **6a** showed a characteristic peak at $\delta = 1.12$ ppm due to the presence of a isopropyl group. Compound **5b** and **6b** showed a triplet at $\delta = 0.95$ and a multiplet at 1.28 ppm due to the presence of the *n*-butyl group. Compounds **5h** and **6h** showed doublet δ value at 2.11 ppm (Ar-CH_3), as well as the doublet at $\delta = 3.83$ ppm (Ar-OCH_3) of compounds **5i** and **6i**. Furthermore, compounds **5a–5i**, and **6a–6i** showed characteristic ^{1}H-NMR spectra confirming the assigned structure.

In the EI-MS spectra, molecular ion [M^+] peaks, appeared with different intensities and confirmed the molecular weights of compounds **5a–5i** and **6a–6i**.

The molecular ion peaks were the base peaks for the compounds; the appearance of an isotope peak [$M^+ + 2$] as intense as the molecular ion peak confirmed the presence of a halogen atom in compounds **5e**, **6e**, **5f**, **6f**, **5g**, and **6g**. The elemental analyses of the compounds **5a–5i** and **6a–6i** were found within the limits of the theoretical values.

Pharmacology

Antihypertensive activity of the synthesized compounds was carried out by non-invasive blood pressure measurement (NIBP). Their results were summarized in Table 1 and Fig. 1. Percentage-inhibition data for non-invasive blood pressure measurement are given in Table 2. Some of the compounds, which show good antihypertensive activity, were further assessed by invasive blood pressure (IBP) measurement [22, 23]. The results for invasive blood pressure measurement are given in Table 3 and Fig. 2. Percentage-inhibition data for non-invasive blood pressure measurement are given in Table 4. Both methods determining the changes in blood pressure were performed by using Power Lab/4SP with ML135 Dual Bio Amp computerized BP monitors automatic cardiovascular system (AD instruments Pvt. Ltd., Australia). The reproducibility of the above experiment was evaluated by using one way ANOVA technique and was found to be statistically significant.

Table 1. Antihypertensive activity data at a 10 mg/kg dose.

Compound	Average blood pressure (in mm Hg) at time (h)										
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
4a	226 ± 4	222 ± 8	220 ± 9	193 ± 2	185 ± 4	175 ± 8	170 ± 6	165 ± 4	161 ± 6	155 ± 3	150 ± 3
4b	226 ± 8	220 ± 5	200 ± 5	193 ± 4	170 ± 5	161 ± 4	161 ± 4	155 ± 6	150 ± 7	140 ± 5	135 ± 8
4c	225 ± 8	220 ± 0	200 ± 5	191 ± 4	170 ± 0	161 ± 7	142 ± 2	135 ± 6	135 ± 5	131 ± 0	130 ± 2
4d	222 ± 3	220 ± 6	210 ± 3	193 ± 5	178 ± 7	161 ± 2	150 ± 5	141 ± 6	138 ± 7	133 ± 5	131 ± 8
4e	228 ± 3	220 ± 6	210 ± 3	183 ± 5	170 ± 7	161 ± 2	150 ± 5	141 ± 6	138 ± 7	135 ± 5	132 ± 8
4f	226 ± 3	220 ± 6	210 ± 3	190 ± 5	175 ± 7	160 ± 2	150 ± 5	138 ± 6	135 ± 7	130 ± 5	130 ± 8
4g	226 ± 2	224 ± 3	210 ± 8	190 ± 4	172 ± 5	160 ± 4	142 ± 4	135 ± 6	130 ± 7	128 ± 5	128 ± 8
4h	226 ± 2	226 ± 3	215 ± 8	195 ± 4	170 ± 5	165 ± 4	140 ± 4	135 ± 6	130 ± 7	120 ± 5	115 ± 8
4i	226 ± 3	221 ± 6	205 ± 3	195 ± 5	175 ± 7	170 ± 2	160 ± 5	145 ± 6	135 ± 7	130 ± 5	125 ± 8
4j	224 ± 3	216 ± 6	205 ± 3	180 ± 5	170 ± 7	155 ± 2	140 ± 5	122 ± 6	120 ± 7	115 ± 5	110 ± 8
4k	226 ± 2	224 ± 3	210 ± 8	190 ± 4	172 ± 5	160 ± 4	142 ± 4	135 ± 6	130 ± 7	125 ± 5	122 ± 8
4l	230 ± 3	220 ± 6	210 ± 3	190 ± 5	175 ± 7	160 ± 2	140 ± 5	125 ± 6	120 ± 7	115 ± 5	110 ± 8
4m	220 ± 5	210 ± 6	200 ± 5	185 ± 1	175 ± 7	160 ± 2	140 ± 5	135 ± 5	135 ± 5	125 ± 5	124 ± 7
4n	225 ± 3	220 ± 5	210 ± 3	190 ± 5	175 ± 7	160 ± 4	145 ± 6	137 ± 7	131 ± 7	131 ± 5	130 ± 8
6a	225 ± 4	222 ± 8	221 ± 9	195 ± 2	180 ± 4	175 ± 8	162 ± 6	155 ± 4	150 ± 6	150 ± 3	140 ± 3
6b	226 ± 8	224 ± 5	210 ± 5	193 ± 4	178 ± 5	161 ± 4	155 ± 4	155 ± 6	150 ± 7	145 ± 5	135 ± 8
6c	226 ± 8	224 ± 5	210 ± 5	193 ± 4	178 ± 5	161 ± 4	153 ± 4	148 ± 6	141 ± 7	135 ± 5	132 ± 8
6d	225 ± 4	222 ± 8	220 ± 9	190 ± 2	178 ± 4	172 ± 8	167 ± 6	160 ± 4	158 ± 6	152 ± 3	145 ± 3
6e	225 ± 6	223 ± 5	210 ± 6	195 ± 8	180 ± 5	160 ± 8	145 ± 3	140 ± 6	135 ± 7	128 ± 5	128 ± 7
6f	226 ± 3	224 ± 6	210 ± 3	193 ± 5	178 ± 7	161 ± 2	142 ± 5	138 ± 6	134 ± 7	130 ± 5	124 ± 8
6g	224 ± 4	222 ± 8	220 ± 9	190 ± 2	175 ± 4	172 ± 8	162 ± 6	160 ± 4	154 ± 6	150 ± 3	145 ± 3
6h	226 ± 2	224 ± 3	210 ± 8	190 ± 4	172 ± 5	160 ± 4	142 ± 4	135 ± 6	130 ± 7	125 ± 5	125 ± 8
6i	225 ± 4	222 ± 8	221 ± 9	195 ± 2	180 ± 4	170 ± 8	168 ± 6	155 ± 4	145 ± 6	135 ± 3	120 ± 3
Control	225 ± 2	224 ± 1	225 ± 1	224 ± 3	224 ± 4	224 ± 1	225 ± 1	224 ± 4	225 ± 2	224 ± 3	225 ± 2
Standard[§]	225 ± 1	214 ± 2	201 ± 1	193 ± 3	182 ± 2	173 ± 1	151 ± 2	131 ± 2	118 ± 1	108 ± 2	103 ± 3

[§] Standard: Valsertan.

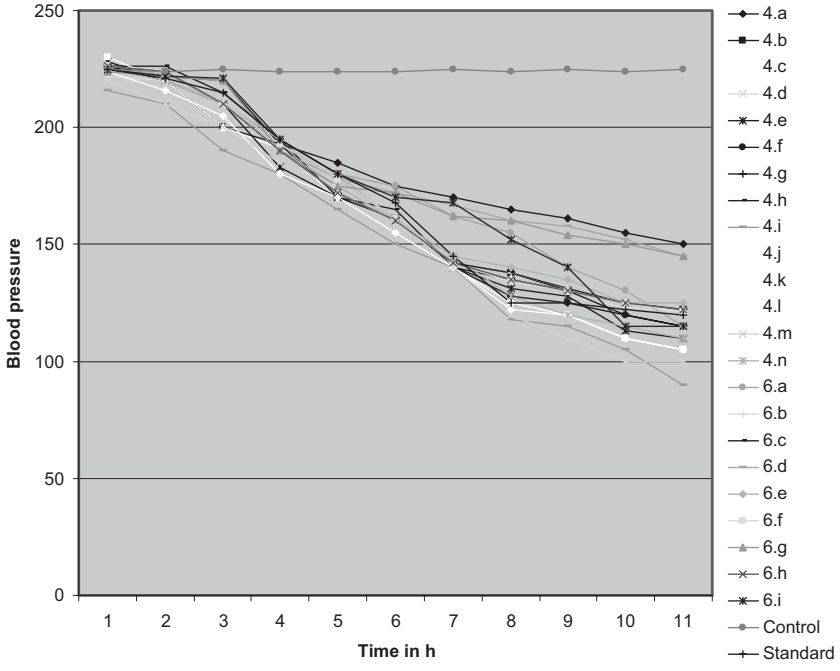


Figure 1. Graph showing decrease in blood pressure compared with standard by non-invasive tail-cuff method.

Table 2. Comparative study of %-inhibition for antihypertensive activity.

Compound	Average blood pressure (in mm Hg) at time (h)										
	0	1	2	3	4	5	6	7	8	9	10
4a	−0.82	0.89	2.2	13.8	17.4	21.8	24.4	26.3	28.4	30.8	33.3
4b	0	2.65	11.5	14.6	24.7	28.7	28.7	31.4	33.6	38.0	40.2
4c	−0.44	1.78	11.1	14.7	24.1	28.1	36.8	39.7	40.2	42.0	42.4
4d	0.89	1.78	6.6	13.8	20.5	28.1	37.7	41.5	43.1	45.0	46.2
4e	−1.78	1.78	6.6	18.3	24.1	28.1	37.7	41.5	43.1	49.5	46.0
4f	−0.89	1.78	6.6	15.1	21.8	28.5	37.7	42.8	44.4	46.4	48.8
4g	−0.89	0	6.6	15.1	23.2	28.5	36.8	39.7	42.2	44.1	45.7
4h	−0.89	−0.89	4.4	12.9	24.1	26.3	37.7	39.7	42.2	46.4	48.8
4i	0	0.88	7.07	25.9	23.8	29.2	37.1	40.2	42.4	44.6	46.0
4j	0	3.57	8.8	19.6	24.1	30.8	37.7	45.5	46.6	50.8	51.3
4k	−0.89	0	6.6	15.1	23.2	28.5	36.8	39.7	42.2	44.1	45.7
4l	−2.6	1.78	6.6	15.1	21.8	28.5	37.7	44.1	46.6	50.8	51.3
4m	1.78	6.25	11.1	17.4	21.8	28.5	38.0	40.2	40.2	44.6	45.5
4n	−0.44	1.78	6.6	15.1	21.8	28.5	35.8	39.3	42.0	42.0	42.4
6a	−0.44	0.89	1.7	12.9	19.6	21.8	28	31.4	33.6	33.6	38.8
6b	−0.89	0	6.6	13.8	20.5	28.1	31.4	31.4	33.6	35.8	40.1
6c	−0.89	0	6.6	13.8	20.5	28.1	36.8	38.3	41.7	44.1	45.6
6d	−0.44	0.89	2.2	15.1	20.5	23.2	25.7	28.5	29.7	32.1	35.5
6e	−0.44	0.44	6.6	12.9	19.6	28.5	35.5	37.5	40	44.1	44.4
6f	−0.89	0	6.6	13.8	20.5	28.1	36.8	40.1	42.2	44.1	46.2
6g	0	0.89	2.2	15.1	21.8	23.2	28	28.5	31.5	33.0	35.5
6h	−0.89	0	6.6	15.1	23.2	28.5	36.8	39.7	42.2	44.1	44.1
6i	−0.44	0.89	1.7	12.9	19.6	24.1	25.3	32.1	37.7	48.6	48.8
Control	–	–	–	–	–	–	–	–	–	–	–
Standard[§]	3.57	5.75	14.5	18.6	28.3	31.0	33.7	45.3	47.8	52.9	54.7

[§] Standard: Valsertan.

3D-QSAR study of the synthesized compounds

The synthesized compounds and their antihypertensive activity data were used to generate 3D-QSAR models in order to gain insights into the structural and molecular properties of these compounds using the PHASE [24–26] program (Schrödinger Computational Chemistry, USA). In general, 3D-QSAR is a useful tool in molecular design and medicinal chemistry, which allows the prediction of the activity of structurally diverse compounds, and also may assists in identifying new molecules with improved activity. The results are summarized in Tables 5 to 7 and in Fig. 3.

Discussion

Synthesis

In the present investigation, different derivatives of (4-hydroxyphenyl)-[5-substituted alkyl/aryl)-2-thioxo-1,3,4-thiadiazol-3-yl]methanone and 4-hydroxy-*N*'-[-3-substituted alkyl/aryl-4-oxo-1,3-thiazolidin-2-ylidene]benzohydrazide were synthesized and their structures were confirmed from their physical and analytical data. The selectivity of the substitution as shown in Schemes 1 and 2 was believed to be due to the electron density at N_1 and N_2. The latter nitrogen

Table 3. Antihypertensive activity of compounds by direct cannulation of carotid artery method (10 mg/kg dose).

Compound (10 mg/kg)	Average blood pressure (in mm Hg) at time (min)						
	0	15	30	60	120	180	240
4g	226 ± 2	216 ± 3	210 ± 8	190 ± 4	165 ± 5	156 ± 4	130 ± 5
4j	224 ± 3	220 ± 6	203 ± 3	180 ± 5	165 ± 7	150 ± 2	125 ± 8
4l	226 ± 2	221 ± 3	216 ± 8	190 ± 4	170 ± 5	156 ± 4	115 ± 8
6f	226 ± 3	218 ± 6	210 ± 3	193 ± 5	168 ± 7	156 ± 2	132 ± 8
6i	225 ± 4	218 ± 8	212 ± 9	195 ± 2	169 ± 4	160 ± 8	135 ± 3
Control	225 ± 2	224 ± 1	225 ± 1	224 ± 3	224 ± 4	224 ± 1	225 ± 7
Standard[§]	225 ± 1	211 ± 2	194 ± 1	189 ± 3	165 ± 2	151 ± 1	124 ± 8

[§] Standard: Valsertan.

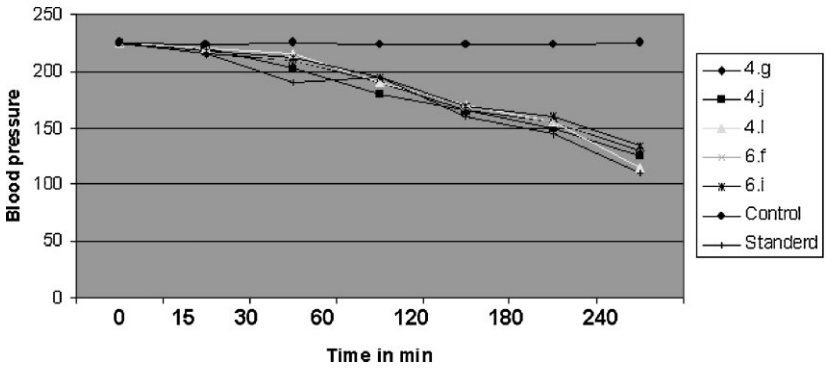


Figure 2. Graph showing decrease in blood pressure compared with standard by direct cannulation of carotid artery.

Table 4. Comparative study of %-inhibition by the method of direct cannulation of the carotid artery (10 mg/kg dose).

Compound (10 mg/kg)	Average blood pressure (in mm Hg) at time (min)						
	0	**15**	**30**	**60**	**120**	**180**	**240**
4g	−0.4	3.5	6.6	15.1	26.3	30.3	42.2
4j	0.4	1.7	9.7	19.6	26.3	33.0	44.4
4l	−0.4	1.3	4	15.1	24.1	30.3	48.8
6f	−0.4	2.6	6.6	13.8	25	30.3	41.3
6i	0	2.6	5.7	12.9	24.5	28.5	40
Control	–	–	–	–	–	–	–
Standard[§]	0	3.5	14.5	18.9	26.5	33.2	50.5

[§] Standard: Valsertan.

Table 5. Data set used for 3D QSAR analysis with corresponding actual and predicted pIC_{50} activity of compounds as antihypertensive agents.

Compound	Antihypertensive activity			Compound	Antihypertensive activity		
	Actual pIC_{50}	**Predicted pIC_{50}**	**Residuals**		**Actual pIC_{50}**	**Predicted pIC_{50}**	**Residuals**
4a	0.30	N.D	N.D	**4m**[§]	0.09	0.06	0.15
4b	0.07	N.D	N.D	**4n**	0.01	0.01	0
4c[§]	0.07	005	−0.02	**6a**	0.02	0.024	0.004
4d	0.06	004	−0.02	**6b**	0.07	0.053	−0.017
4e[§]	0.01	001	0	**6c**	0.07	0.07	0
4f	0.02	002	0	**6d**	0.25	0.23	−0.02
4g	0.07	005	−0.02	**6e**	0.09	0.07	−0.02
4h	0.02	001	−0.01	**6f**	0.06	0.03	−0.03
4i[§]	0.17	010	0.07	**6g**	0.25	0.05	−0.2
4j	0.05	004	0.09	**6h**	0.074	0.05	−0.024
	0.05	001	0.06	**6i**[§]	0.02	0.07	0.05
	0.07	002	−0.05				

[§] Testset compounds; N.D: values not obtained.

Table 6. QSAR hypothesis score.

ID	Survival	Survival -inactive	Post-hoc	Site	Vector	Volume	Selectivity	# Matches	Energy	Activity	Inactive
ADHR.12[√]	**38.835**	**32.097**	**3.72**	**0.95**	**0.931**	**0.885**	**2.202**	**37**	**0**	**4.638**	**1.737**
AAHR.28	39.791	34.164	3.516	0.85	0.862	0.804	2.362	37	0.01	4.42	1.627
ADRR.32	29.208	27.696	3.675	0.91	0.92	0.849	2.42	33	5.191	4.638	1.512
AARR.40	32.206	32.702	3.67	0.91	0.909	0.847	2.422	33	5.169	4.602	1.504

[√] : optimum model.

Table 7. PHASE 3D-QSAR statistical analysis.

Pharmacophore	Model 1	Model 2	Model 3	Model 4
Hypothesis	ADHR$^{\surd}$	AAHR	ADRR	AARR
PLS & statistics for QSAR model				
r^2	0.98	0.94	0.84	0.92
SD [§]	0.05	0.03	0.02	0.04
F	54.1	27.1	43.2	28.1
P	$1.41e^{12}$	$4.22e^{13}$	$2.56e^{12}$	$3.22e^{13}$
No of PLS [&] factors	3	3	3	3
External test set for prediction				
q^2	0.74	0.48	0.54	0.45
r_P	0.91	0.82	0.81	0.90
RMSE	0.1323	0.1321	0.1322	0.1222

[§] SD: standard deviation; & PLS: partial least square; $^{\surd}$ optimum model.

being richer in electron density is more reactive and provides products of exclusive functionalization at N_2 [11].

Antihypertensive activity

Antihypertensive activity testing for the compounds synthesized according to Schemes 1 and 2 was carried out by the non-invasive method (NIBP). In Scheme 1, 2,6 dichlorophenyl compound **4g**, 4-methoxyphenyl compound **4j**, and 3,4-disubstituted methoxyphenyl compound **4l** had a remarkable antihypertensive activity at 10 mg/kg. Although, 3-hydroxy-4-methoxyphenyl compound **4k** is slightly less potent than the corresponding compounds **4g**, **4j**, and **4l**. The other compounds showed moderate activity. From Scheme 2, 2,4-dichlorophenyl compound **6f** and 2,4-dimethoxyphenyl compound **6i** showed remarkable antihypertensive activity. The other compounds synthesized according to Scheme 2 showed only moderate activity. It was observed that electron-withdrawing or electron-donating substitutions may impart the lipophilicity and show a better activity. The data are presented as means ± S.E.M.; a repeated measures analysis of variance was used to obtain the statistical significance between and within the groups. Differences were considered statistically significant at $P < 0.05$, and the F values for all compounds in Schemes 1 and 2 are 80.33 ± 0.5. The results of %-inhibition are shown in Table 2. Few compounds showed remarkable antihypertensive activity; these are compounds **4g**, **4j**, **4l**, **6f**, and **6i**. They were further tested by the invasive method (IBP; direct cannulation of carotid artery).

In the invasive method, the 4-methoxyphenyl compound **4j** showed a remarkable antihypertensive activity and the 3,4-disubstituted methoxyphenyl compound **4l** had good antihypertensive activity.

From the ANOVA analysis and %-inhibition studies, it was concluded that the substitution (R) with phenyl/substituted-phenyl showed a better activity than substitution (R) with an aliphatic group/furan.

3D-QSAR study

A 3D-QSAR study was performed for compounds **4a–4n** and compounds **6a–6i** to generate 3D-QSAR models. The statistically significant 3D-QSAR models were generated using 17 compounds as training set and were validated using a test set with six compounds (Table 5) employing PHASE, the pharmacophore modeling tool. A total of 101 five-point hypotheses were obtained upon completion of the scoring process and the top four high-ranking pharmacophore hypotheses were analyzed. The statistical and partial least square (PLS) analyses results for four of the top ranking pharmacophores (model 1–4) are summarized in Table 7. Model 1 consisted of a hydrogen bond acceptor (A), one hydrogen bond donor (D), a hydrophobic group (H), and one aromatic ring (R). Model 2 was composed of two hydrogen-bond acceptors (A), one hydrophobic group (H), and one aromatic ring (R). Model 3 consisted of one hydrogen-bond acceptor (A), one hydrogen bond donor (D), and two aromatic rings (R). Model 4 possessed two hydrogen-bond acceptor (A) and two aromatic rings (R). Model 1 exhibited comparatively better PLS statistical qualities and excellent prediction of the external test set compounds, and was, therefore, considered for explaining the SAR of the compounds. Model 1 showed a conventional correlation coefficient (r^2) of 0.98 with three components, test set prediction (q^2) of 0.74 and Pearson-R (r_P) of 0.91. The high value of variance ratio (F) observed for this model indicates its statistical robustness which is further supported by the significance level of the variance ratio (P). The value of $P < 0.05$ indicates a greater degree of confidence and means F is significant at the 95% level. The low standard deviation (SD) and root-mean-squared error (RMSE) contributes significantly to the model. The common pharmacophore generated from the best PHASE hypothesis with aligned compound **4j** is shown in Fig. 3a. It shows pharmacophoric features: black sphere for the

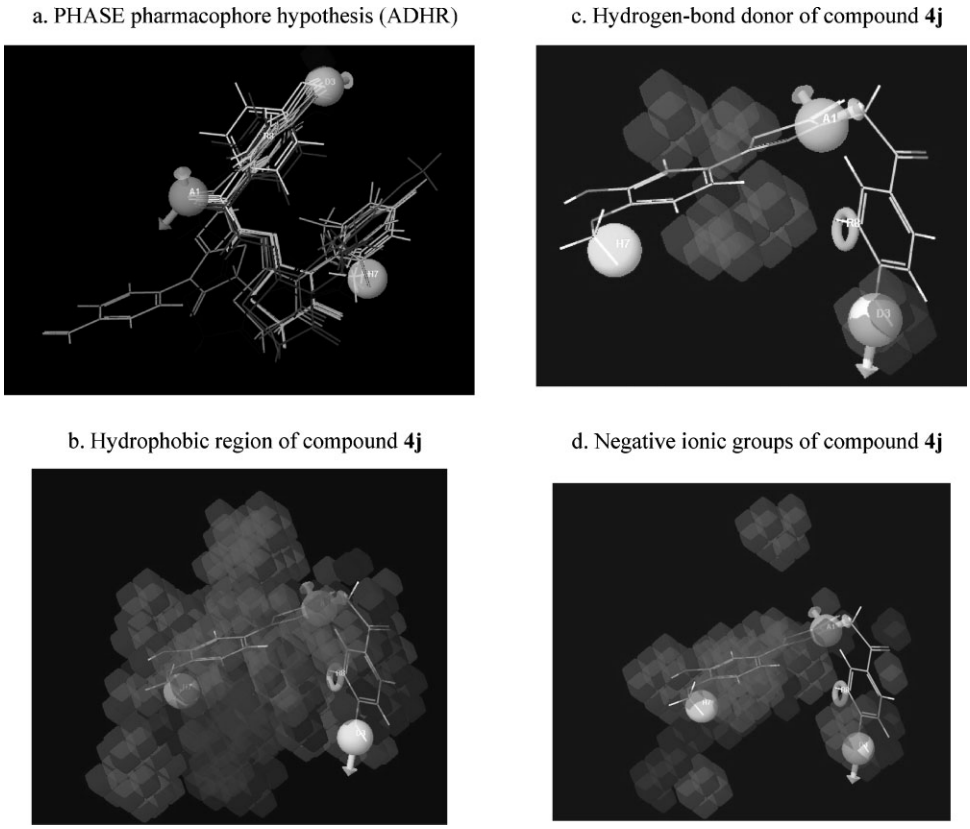


Figure 3. Visual representation of atom-based PHASE QSAR. PHASE-3D plots of the crucial pharmacophore regions based on the model displayed with compound **4j**. Positive coefficient-favored areas (contributing for increase in activity) are represented by gray cubes. Negative coefficient-favored areas (contributing for decrease in activity) are represented by black cubes.

hydrogen-bond acceptor (A) with the arrows pointing in the direction of lone pair, gray sphere for the donor group (D), and the torus for aromatic rings (R). In addition, the 3D-QSAR results are also visualized as 3D plots of crucial pharmacophore regions in which the gray cubes (regions) indicate increase – and black cubes (regions) indicate decrease – in activity for specific feature in the ligand regions.

Figures 3b to 3d show the 3D-QSAR plots with specific features relating to the most active conformer overlaid with the best-generated pharmacophore hypothesis (compound **4j**) and their relation with the biological activity of the compounds as antihypertensive agents by generating volume-occupied maps.

In the hydrogen-bond donor plots (Fig. 3c), the gray regions at the 4-position of the benzohydrazide ring show the significance of protons in the hydrogen-bond formation with the receptor surface (compounds **4j**, **4i**, and **6i**). The black regions in the vicinity of 3′- and 4′-position of the phenyl ring of *p*-hydroxybenzohydrazide suggested that the substructure fragments with hydrogen-bond donor in this area may reduce the activity (compounds **4a**, **4b**, **6d**, and **6g**). In the negative ionic plots (Fig. 3d), gray regions at the 4′-position of the 5-phenyl ring suggest that increased activity may be anticipated by moderate negatively charged substituents at the 4′-position (compounds **4h** and **4j**), whereas black region surrounding the 6-position of the benzohydrazide ring disfavor the activity (compound **6h**). The hydrophobic (nonpolar) plot indicates desired/undesired hydrophobic regions around two phenyl rings (Fig. 3b). The gray region observed in the vicinity of the OCH_3 group and the 4′-position of the benzohydrazide ring signifies that substitution with a hydrophobic group may result in enhanced activity (compounds **4j** and **4i**), whereas a slight variation in the phenyl ring results in decreased activity (compounds **6a** and **6b**). The disfavored black region in the vicinity of the 4′-position of the 5-phenyl ring suggests that a hydrophobic group in this area results in decreased activity (compounds **6d** and **6h**).

This study demonstrates that the activity may be increased by placing electron-releasing group(s) such as methoxyphenyl/dimethoxyphenyl on the *p*-hydroxybenzohydrazide ring. Moreover, aromatic substitution should be retaining for a better activity. Also, activity may be increase by placing the hydrophobic groups at a particular position of the phenyl ring of the *p*-hydroxybenzohydrazide.

Experimental

Chemistry

Chemicals were obtained from Fluka Chemical Co. (Germany). Melting points (m. p.) were detected with open capillaries using Thermonik Precision Melting point cum Boiling point apparatus (C-PMB-2, Mumbai, India) and are uncorrected. IR spectra (KBr) were recorded on FTIR-8400s spectrophotometer (Shimadzu, Japan). ^{1}H-NMR spectra were obtained using a Varian EM 390 spectrophotometer (Varian, USA) using $CDCl_3$. All chemical-shift values were recorded as δ (ppm). The purity of compounds was controlled by thin layer chromatography (silica gel, $HF_{254\text{-}361}$, type 60, 0.25 mm, Merck, Darmstadt, Germany). Elementary analysis was performed at RTM Nagpur University, India. Elementary analyses for C. H, N were within $\pm$0.4% of theoretical values.

Specific examples presented below illustrate the general synthetic procedures.

*Synthesis of **2***

A mixture of compound **1** (1.5 g, 0.02 mol) and hydrazine hydrate (80%) (4.12 mL, 0.08 mol) was refluxed for 12 h. The excess solvent was removed under reduced pressure and the reaction mixture was cooled to 4–5°C. The solid crystals which separated were filtered, washed with cold water, dried, and recrystallized from ethanol to obtain a white product **2** (1.4 g, m. p.: 172–173°C). Yield: 1.2 g (80%); m. p.: 170–171°C (ethanol/water); R_f: 0.62 (acetonitrile/methanol 1:1); IR (KBr), in cm^{-1}: 3351 (alcohol O-H & C-O stretching), 3013 (Ar-H stretching), 1622 (C-O stretching), 1185, 1034 (alcohol O-H stretching), 832 (benzene 1,4 disubstituted); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.5 (s, 1H, CONH), 5.32 (s, 2H, NH_2); EI-MS (*m*/*z*, 100%): 152 [M + 2] (100). Anal. calcd. for $C_7H_8N_2O_2$: C, 55.26; H, 5.31; N, 18.40. Found: C, 55.26; H, 5.30; N, 18.46.

*General procedure for synthesis of **3a–3n***

A solution of the corresponding compound **2** (1.5 g 10 mmol) in ethanol (40 mL) was refluxed with various aliphatic/aromatic aldehydes (10 mmol) for 3 h. The excess of solvent was removed under reduce pressure. After cooling to room temperature, a white solid appeared. This crude product was filtered, washed with diethyl ether, dried, and recrystallized from rectified spirit.

The physical and analytical data of some selected compounds are given below

*Compound **3a***

A solution of the corresponding compound **2** (1.5 g, 10 mmol) in ethanol (40 mL) was refluxed with propenaldehyde (0.4 g, 10 mmol) for 3 h. The excess of solvent was removed under reduce pressure. After cooling to room temperature, a white solid appeared. This crude product was filtered, washed with diethyl ether, and recrystallized from methanol affording the desired white product **3a**. Yield: 1.17 g (78%); m. p.: 123–124°C (rectified spirit); R_f: 0.59 (acetonitrile/methanol 1:1); IR (KBr), in cm^{-1}: 3090 (Ar-OH, NH), 1672–1682 (hydrazide –C=O), 1500–1569 (–C=N), 2986, 2990 (CH(CH_3)$_2$ stretching); ^{1}H-NMR (DMSO-d_6) δ: 2.09 (s, 1H, CH_3), 4.00 (m, 1H, isopropyl CH), 4.27 (s, 1H, NCH_2), 4.32 (s, 1H, $NHNH_2$), 5.24 (s, 2H, Ar-OH), 8.42 (s, 1H, CH-N), 12.13 (d, 2H, CONHN=CH); EI-MS (*m*/*z*, 100%): 206 [M + 2] (100). Anal. calcd. for $C_{11}H_{14}N_2O_2$: C, 64.08; H, 6.84; N, 13.57. Found: C, 64.06; H, 6.84; N, 13.58.

*Compound **3c***

A solution of the corresponding compound **2** (1.5 g, 10 mmol) in ethanol (40 mL) was refluxed with benzaldehyde (1.06 g, 10 mmol) affording the white product **3c** by following the procedure as described for **3a** (refluxing for 4 h). Yield: 1.14 g (76%); m. p.: 172–173°C (rectified spirit); R_f: 0.7 (acetonitrile/methanol 1:1); IR (KBr), in cm^{-1}: 3120–3221 (Ar-OH, NH), 1668–1682 (hydrazide –C=O), 1569 (C-Ar stretching), 1510–1582 (–C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.09 (s, 1H, CH_3), 4.27 (s, 1H, NCH_2), 4.32 (s, 1H, $NHNH_2$), 5.31 (s, 1H, Ar-OH), 8.42 (s, 1H, CH-N),/11.95 (d, 2H, CONHN=CH), 6.1–7.5 (m, 4H, Ar-H); EI-MS (*m*/*z*, 100%): 240 [M + 2] (100). Anal. calcd. for $C_{14}H_{12}N_2O_2$: C, 69.96; H, 5.02; N, 11.65. Found: C, 69.99; H, 5.03; N, 11.66.

*Compound **3d***

A solution of the corresponding compound **2** (1.5 g, 10 mmol) in ethanol (40 mL) was refluxed with 4-nitrobenzaldehyde (1.6 g, 10 mmol) afforded the yellowish product **3d** by following the procedure as described for **3a** (refluxing for 5 h). Yield: 1.0 g (67%); m. p.: 189–190°C (rectified spirit); R_f: 0.7 (acetonitrile/methanol 1:1); IR (KBr), in cm^{-1}: 3180–3230 (Ar-/OH, NH), 1668–1682 (hydrazide –C=O), 1487–1555 (C-NO_2 stretching), 1578 (C-Ar stretching), 1520–1582 (–C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.09 (s, 3H, CH_3), 4.27 (s, 1H, NCH_2), 5.29 (s, 1H, Ar-OH), 8.22 (s, 1H, CH-N), 11.78 (d, 2H, CONHN=CH); EI-MS (*m*/*z*, 100%): 285 [M + 2] (100). Anal. calcd. for $C_{14}H_{11}N_3O_4$: C, 58.96; H, 3.89; N, 14.70. Found: C, 59.96; H, 3.85; N, 14.73.

*Compound **3e***

A solution of the corresponding compound **2** (1.5 g, 10 mmol) in ethanol (40 mL) was refluxed with 4-chlorobenzaldehyde (1.4 g, 10 mmol) affording the desired white product **3e** by following the procedure as described for **3a** (refluxing for 5 h). Yield: 1.2 g (81%); m. p.: 165–166°C (rectified spirit); R_f: 0.56 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3200–3280 (Ar-OH, NH), 1658–1684 (hydrazide –C=O), 1080–1092 (Ar-Cl stretching), 1578 (C-Ar stretching), 1520–1582 (–C=N); ^{1}H-NMR (DMSO-d_6) δ: 4.27 (s, 2H, NCH_2), 5.29 (s, 1H, Ar-OH), 8.22 (s, 1H, CH-N), 11.78 (s, 2H, CONHN=CH), 7.35–8.01 (m, 8H, ArH); EI-MS (*m*/*z*, 100%): 274.29 [M + 2] (100). Anal. calcd. for $C_{14}H_{11}ClN_2O_2$: C, 61.16; H, 4.09; N, 10.22. Found: C, 61.21; H, 4.04; N, 10.20.

*Compound **3f***

A solution of the corresponding compound **2** (1.5 g, 10 mmol) in ethanol (40 mL) was refluxed with 4-fluorobenzaldehyde (1.24 g, 10 mmol) affording the desired product **3f** (1.3 g, m. p.: 173–174°C) by following the procedure as described fort **3a** (refluxing for 6 h). Yield: 1.1 g (76%); m. p.: 173–174°C (rectified sprit); R_f: 0.7 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3280–3320 (Ar-OH, NH), 1658–1684 (hydrazide –C=O), 3010–3030

(Ar-CH stretching), 1578 (C-Ar stretching), 1621–1633 (–C=N); ^{1}H-NMR (DMSO-d_6) δ: 4.12 (s, 2H, NCH_2), 4.31 (s, 1H, $NHNH_2$), 5.40 (s,1H, NH_2), 11.04 (s, 2H, CONHN=CH), 7.55–8.01 (m, 8H, ArH); EI-MS (*m/z*, 100%): 254.31 [M + 2] (100). Anal. calcd. for $C_{15}H_{14}N_2O_2$: C, 70.80; H, 5.51; N, 11.01. Found: C, 70.83; H, 5.52; N, 11.02.

*Compound **3h***

A solution of the corresponding compound **2** (1.5 g, 10 mmol) in ethanol (40 mL) was refluxed with 4-methylbenzaldehyde (1.2 g, 10 mmol) affording the desired white product **3h** by following the procedure as described for **3a** (refluxing for 7 h). Yield: 1.03 g (69%); m. p.: 181–182°C (rectified spirit); R_f: 0.68 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3266–3380 (Ar-OH, NH), 1658–1684 (hydrazide –C=O), 1578 (C-Ar stretching), 1621–1633 (–C=N); ^{1}H-NMR (DMSO-d_6) δ: 4.02 (s, 2H, NCH_2), 4.17 (s, 1H, $NHNH_2$), 5.45 (s, 1H, Ar-OH), 11.24 (s, 2H, CONHN=CH), 7.55–8.01 (m, 8H, ArH); EI-MS (*m/z*, 100%): 267.34 [M + 2] (100). Anal. calcd. for $C_{14}H_{12}N_2O_3$: C, 51.99; H, 3.45; N, 13.04. Found: C, 52.01; H, 3.43; N, 13.00.

*Compound **3i***

A solution of the corresponding compound **2** (1.5 g, 10 mmol) in ethanol (40 mL) was refluxed with 4-hydroxybenzaldehyde (1.25 g, 10 mmol) affording the desired whitish product **3i** by following the procedure as described for **3a** (refluxing for 6 h). Yield: 1.09 g (73%); m. p.: 156–157°C (rectified spirit); R_f: 0.65 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3276–3390 (Ar-OH, NH), 1050–1150 (O-CH_3 stretching), 1658–1682 (hydrazide –C=O), 1568 (C-Ar stretching), 1610 (–C=N); ^{1}H-NMR (DMSO-d_6) δ: 1.21 (t, 3H, OCH_3-C_6H_4), 5.27 (s, 1H, Ar-OH), 8.22 (s, 1H, CH-N), 11.78 (2H, CONHN=CH); EI-MS (*m/z*, 100%): 270 [M + 2] (100). Anal. calcd. for $C_{15}H_{14}N_2O_3$: C, 66.61; H, 5.22; N, 10.30. Found: C, 66.66; H, 5.22; N, 10.36.

*Compound **3j***

A solution of the corresponding compound **2** (1.5 g, 10 mmol) in ethanol (40 mL) was refluxed with 4-methoxybenzaldehyde (1.36 g, 10 mmol) affording the desired white product **3j** by following the procedure as described for **3a** (refluxing for 6 h). Yield: 0.96 g (64%); m. p.: 165–166°C (rectified spirit); R_f: 0.65 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3276–3390 (Ar-OH, NH), 1654–1684 (hydrazide –C=O), 1568 (C-Ar stretching), 1610 (–C=N); ^{1}H-NMR (DMSO-d_6) δ: 2.35 (d, 6H, CH_3), 4.22 (s, N-CH), 8.10 (s, 1H, CH-N), 11.97 (2H, CONHN=CH); EI-MS (*m/z*, 100%): 283 [M + 2] (100). Anal. calcd. for $C_{16}H_{17}N_3O_2$: C, 67.81; H, 6.02; N, 14.81. Found: C, 67.83; H, 6.05; N, 14.83.

*General procedure for synthesis of compounds **4a–4n***

To a mixture of the corresponding compounds **3a–3n** (0.01 mol) in ethanol (50 mL), a solution of potassium hydroxide (0.01 mol) in ethanol (10 mL) was added followed by carbon disulphide (20 mL). The reaction mixture was heated under refluxed for 6 h. It was concentrated and poured into crush ice. The resultant solid was filtered, dried, and recrystallized from a mixture of DMF and water (1:1).

*Compound **4a***

To a solution of compound **3a** (1.9 g, 0.01 mol) in ethanol (50 mL), a mixture of potassium hydroxide (0.54 g, 0.01 mol) in ethanol (10 mL) and carbon disulphide (20 mL) was added. The reaction mixture was heated under reflux for 6 h. It was concentrated and poured into crush ice. The resultant solid was filtered, dried, and recrystallized to give the white product **4a**. Yield: 1.15 g (61%); m. p.: 177–178°C (DMF/water); R_f: 0.69 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 1189-1290 (C=S stretching), 1689–1720 (–C=O), 1568 (C-Ar stretching); ^{1}H-NMR (DMSO-d_6) δ: 1.31–1.53 (m, 4H, CH-$(CH_3)_2$), 2.97 (s, 3H, CH_3–N), 5.23 (s, 1H, Ar-OH), 8.10 (s, 1H, CH-N); EI-MS (*m/z*, 100%): 280 [M + 2] (100). Anal. calcd. for $C_{12}H_{12}N_2O_2S_2$: C, 50.41; H, 4.21; N, 9.92. Found: C, 51.41; H, 4.31; N, 9.99.

*Compound **4c***

To a solution of compound **3c** (2.4 g, 0.01 mol) in ethanol (50 mL), a mixture of potassium hydroxide (0.54 g, 0.01 mol) in ethanol (10 mL) and carbon disulphide (20 mL) was added. The reaction mixture was heated under reflux for 8 h. It was concentrated. Following the procedure described for **4a** gave the white product **4c**. Yield: 1.7 g (71%); m. p.: 191–192°C (DMF/water); R_f: 0.56 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 1191–1240 (C=S stretching), 1680–1710 (–C=O), 1577 (C-Ar stretching); ^{1}H-NMR (DMSO-d_6) δ: 5.23 (s, 1H, Ar-OH), 7.0–8.5 (m, 9H, Ar-H); EI-MS (*m/z*, 100%): 314 [M + 2] (100). Anal. calcd. for $C_{15}H_{10}N_2O_2S_2$: C, 57.28; H, 3.20; N, 8.90. Found: C, 57.31; H, 3.21; N, 8.91.

*Compound **4d***

To a solution of compound **3d** (2.8 g, 0.01 mol) in ethanol (50 mL), a mixture of potassium hydroxide (0.54 g, 0.01 mol) in ethanol (10 mL) and carbon disulphide (20 mL) was added. The reaction mixture was heated under reflux for 7 h. It was concentrated. Following the procedure described for **4a** afforded the yellowish product **4d**. Yield: 1.7 g (62%); m. p.: 237–238°C (DMF/water); R_f: 0.66 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 1191–1240 (C=S stretching), 1680–1720 (–C=O), 1487 (Ar-NO_2 stretching), 1577 (C-Ar stretching); ^{1}H-NMR (DMSO-d_6) δ: 5.33 (s, 1H, Ar-OH), 6.88–7.86 (m, 4H, Ar-H_1) 8.09–8.33 (m, 4H, Ar-H_2); EI-MS (*m/z*, 100%): 359 [M + 2] (100). Anal. calcd. for $C_{15}H_9N_3O_4S_2$: C, 50.11; H, 2.41; N, 11.57. Found: C, 50.13; H, 2.52; N, 11.69.

*Compound **4e***

To a solution of compound **3e** (2.7 g, 0.01 mol) in ethanol (50 mL), a mixture of potassium hydroxide (0.54 g, 0.01 mol) in ethanol (10 mL) and carbon disulphide (20 mL) was added. The reaction mixture was heated under reflux for 8 h. It was concentrated. Following the procedure described for **4a** afforded the white product **4e**. Yield: 2.2 g (80%); m. p.: 237–238°C (DMF/water); R_f: 0.59 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 1191–1240 (C=S stretching), 1092–1100 (Ar-Cl stretching), 1680–1720 (–C=O), 1577 (C-Ar stretching); ^{1}H-NMR (DMSO-d_6) δ: 5.22 (s, 1H, Ar-OH), 6.88–7.78 (m, 4H, Ar-H_1), 7.52–7.77 (m, 4H, Ar-H_2); EI-MS (*m/z*, 100%): 348 [M + 2] (100). Anal. calcd. for $C_{15}H_9ClN_2O_2S_2$: C, 51.61; H, 2.53; N, 9.96. Found: C, 51.65; H, 2.60; N, 10.16.

*Compound **4h***

To a solution of compound **3h** (2.5 g, 0.01 mol) in ethanol (50 mL), a mixture of potassium hydroxide (0.54 g, 0.01 mol) in ethanol (10 mL) and carbon disulphide (20 mL) was added. The reaction mixture was heated under reflux for 8 h. It was concentrated. Following the procedure described for **4a** afforded

the whitish product **4h**. Yield: 2.1 g (79%); m. p.: 212–213°C (DMF/water); R_f: 0.67 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 1191–1240 (C=S stretching), 1670–1730 (–C=O), 1577 (C-Ar stretching); ^{1}H-NMR (DMSO-d_6) δ: 2.2 (s, 3H, CH_3), 5.12 (s, 1H, Ar-OH), 6.88–7.78 (m, 4H, Ar-H_1), 7.21–7.71 (m, 4-H, Ar-H); EI-MS (*m*/*z*, 100%): 328 [M + 2] (100). Anal. calcd. for $C_{16}H_{12}N_2O_2S_2$: C, 58.51; H, 3.50; N, 8.49. Found: C, 58.52; H, 3.68; N, 8.53.

*Compound **4i***

To a solution of compound **3i** (2.5 g, 0.01 mol) in ethanol (50 mL), a mixture of potassium hydroxide (0.54 g, 0.01 mol) in ethanol (10 mL) and carbon disulphide (20 mL) was added. The reaction mixture was heated under reflux for 7 h. It was concentrated. Following the procedure described for **4a**, afforded the whitish product **4i**. Yield: 1.9 g (79%); m. p.: 218–219°C (DMF/water); R_f: 0.77 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 1191–1240 (C=S stretching), 1670–1730 (–C=O), 3600–3400 (Ar-OH), 1577 (C-Ar stretching); ^{1}H-NMR (DMSO-d_6) δ: 5.35 (s, 1H, Ar-OH), 6.88–7.78 (m, 4H, Ar-H), 7.01–7.85 (m, 4H, Ar-H); EI-MS (*m*/*z*, 100%): 330 [M + 2] (100). Anal. calcd. for $C_{15}H_{10}N_2O_3S_2$: C, 54.51; H, 3.02; N, 8.39. Found: C, 54.53; H, 3.05; N, 8.48.

*Compound **4j***

To a solution of compound **3j** (2.7 g, 0.01 mol) in ethanol (50 mL), a mixture of potassium hydroxide (0.54 g, 0.01 mol) in ethanol (10 mL) and carbon disulphide (20 mL) was added. The reaction mixture was heated under reflux for 6 h. It was concentrated. Following the procedure described for **4a** afforded the whitish product **4j**. Yield: 1.9 g (74%); m. p.: 202–203°C (DMF/water); R_f: 0.67 (acetonitrile/methanol, 1:1); IR (KBr) [cm^{-1}]: 1191–1240 (C=S stretching), 1670–1730 (–C=O), 3600–3400 (Ar-OH), 1577 (C-Ar stretching); ^{1}H-NMR (DMSO-d_6) δ: 1.2–1.31 (s, 3H, OCH_3), 5.26 (s, 1H, Ar-OH), 6.88–7.78 (m, 4H, Ar-H_1), 7.10–7.71 (m, 4H, Ar-H_2); EI-MS (*m*/*z*, 100%): 343 [M + 2] (100). Anal. calcd. for $C_{16}H_{12}N_2O_3S_2$: C, 55.72; H, 3.50; N, 8.10. Found: C, 55.80; H, 3.51; N, 8.13.

*Compound **4m***

To a solution of compound **3m** (2.8 g, 0.01 mol) in ethanol (50 mL), a mixture of potassium hydroxide (0.54 g, 0.01 mol) in ethanol (10 mL) and carbon disulphide (20 mL) was added. The reaction mixture was heated under reflux for 10 h. It was concentrated. Following the procedure described for **4a** afforded the white product **4m**. Yield: 2.2 g (74%); m. p.: 209–210°C (DMF/water); R_f: 0.56 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 1181–1230 (C=S stretching), 1680–1740 (–C=O), 3600–3400 (Ar-OH), 1577 (C-Ar stretching); ^{1}H-NMR (DMSO-d_6) δ: 4.2–4.4 (s, 6H, $N(CH_3)_2$), 7.2–7.78 (m, 4H, Ar-H_1), 7.65–7.8 (m, 4H, Ar-H_2), 5.21 (s, 1H, -OH); EI-MS (*m*/*z*, 100%): 357 [M + 2] (100). Anal. calcd. for $C_{17}H_{15}N_3O_2S_2$: C, 57.11; H, 4.20; N, 11.74. Found: C, 57.12; H, 4.23; N, 11.76.

*General procedure for synthesis of **5a–5i***

To a solution of **2** (0.01 mol) in ethanol (50 mL), various aliphatic/aromatic isothiocyanates (0.01 mol) were added. The reaction mixture was refluxed for 12 h. Excess solvent was removed under vacuum. The residue was washed with diethyl ether and recrystallized using methanol.

*Compound **5a***

To a solution of compound **2** (1.5 g 0.01 mol) in ethanol (50 mL), isopropyl isothiocyanate (1.01 g, 0.01 mol) was added. The reaction mixture was refluxed for 12 h. Excess solvent was removed under vacuum. The residue was washed with diethyl ether and recrystallized to obtain the white product **5a**. Yield: 1.0 g (70%); m. p.: 225–226°C (methanol); R_f: 0.67 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3213, 3229 (NH stretching), 2986, 2990 ($CH(CH_3)_2$ stretching), 1732 (C=O stretching), 1315 (C=S stretching); ^{1}H-NMR ($CDCl_3$) δ: 1.2 (d, 6H, isopropyl CH_3), 4.00 (m, 1H, isopropyl CH), 7.74 (s, 1H, CONH), 7.2–7.78 (m, 4H, Ar-H), 5.21 (s,1H, -OH); EI-MS (*m*/*z*, 100%): 253 [M + 2] (100). Anal. calcd. for $C_{11}H_{15}N_3O_2S$: C, 52.13; H, 5.98; N, 16.59. Found: C, 52.15; H, 5.97; N, 16.59.

*Compound **5b***

To a solution of compound **2** (1.5 g 0.01 mol) in ethanol (50 mL), *n*-butyl isothiocyanate (1.15 g, 0.01 mol) was added. The reaction mixture was refluxed for 12 h. Following the procedure as described for **5a** afforded the white product **5b**. Yield: 1.1 g (74%); m. p.: 168–169°C (methanol); R_f: 0.66 (acetonitrile/methanol, 1:1); IR (KBr) [cm^{-1}]: 3213, 3224 (NH stretching), 1731 (C=O stretching), 1316 (C=S stretching); ^{1}H-NMR ($CDCl_3$) δ: 0.95 (t, 3H, butyl CH_3), 1.28 (m, 2H, butyl CH_2), 1.51 (m, 2H, butyl CH_2), 3.53 (m, 2H, butyl CH_2), 7.73 (s, 1H, CONH), 6.8–7.78 (m, 4H, Ar-H), 5.3 (s, 1H, -OH); EI-MS (*m*/*z*, 100%): 267 [M + 2] (100). Anal. calcd. for $C_{12}H_{17}N_3O_2S$: C, 53.83; H, 6.41; N, 15.69. Found: C, 53.91; H, 6.41; N, 15.72.

*Compound **5c***

To a solution of compound **2** (1.5 g, 0.01 mol) in ethanol (50 mL), phenyl isothiocyanate (1.35 g, 0.01 mol) was added. The reaction mixture was refluxed for 12 h. Following the procedure as described for **5a** afforded the white product **5c**. Yield: 1.2 g (82%); m. p.: 175–176°C (methanol); R_f: 0.66 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3217, 3232 (NH), 1738 (C=O stretching), 1313 (C=S stretching); ^{1}H-NMR ($CDCl_3$) δ: 5.35 (s, 1H, Ar-OH), 6.8–7.62 (m, 9H, Ar-H), 7.75 (s, 1H, CONH); EI-MS (*m*/*z*, 100%): 287 [M + 2] (100). Anal. calcd. for $C_{14}H_{13}N_3O_2S$: C, 58.53; H, 4.51; N, 14.59. Found: C, 58.20; H, 4.56; N, 14.62.

*Compound **5d***

To a solution of compound **2** (1.5 g, 0.01 mol) in ethanol (50 mL), 4-nitrophenyl isothiocyanate (1.8 g, 0.01 mol) was added. The reaction mixture was refluxed for 10 h. Following the procedure as described for **5a** afforded the yellowish product **5d**. Yield: 0.8 g (56%); m. p.: 189–190°C (methanol); R_f: 0.66 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3215, 3230 (NH), 1731 (C=O stretching), 1315 (C=S stretching); ^{1}H-NMR ($CDCl_3$) δ: 5.33 (s, 1H, Ar-OH), 6.88–7.82 (m, 8H, ArH), 7.78 (s, 1H, CONH), 10.40 (s, 1H, NH), 11.81 (s, 1H, NHAr); EI-MS (*m*/*z*, 100%): 322 [M + 2] (100). Anal. calcd. for $C_{14}H_{12}N_4O_4S$: C, 50.59; H, 3.64; N, 16.83. Found: C, 50.60; H, 3.64; N, 16.86.

*Compound **5e***

To a solution of compound **2** (1.5 g, 0.01 mol) in ethanol (50 mL), 4-fluorophenyl isothiocyanate (1.5 g, 0.01 mol) was added. The reaction mixture was refluxed for 11 h. Following the procedure as described in **5a** afforded the white product **5e**. Yield: 1.2 g

(82%), m. p.: 192–193°C (methanol); R_f: 0.7 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3217, 3232 (NH), 1738 (C=O stretching), 1313 (C=S stretching); ^{1}H-NMR ($CDCl_3$) δ: 5.35(s, 1H, Ar-OH), 6.78–7.82 (m, 8H, ArH), 7.78 (s, 1H, CONH), 10.40 (s, 1H, NH), 11.81 (s, 1H, NHAr); EI-MS (*m/z*, 100%): 305 [M + 2] (100). Anal. calcd. for $C_{14}H_{12}FN_3O_2S$: C, 55.07; H, 3.94; N, 13.74. Found: C, 55.07; H, 3.96; N, 13.76.

*Compound **5f***

To a solution of compound **2** (1.5 g, 0.01 mol) in ethanol (50 mL), 2,4-dichlorophenyl isothiocyanate (2.0 g, 0.01 mol) was added. The reaction mixture was refluxed for 12 h. Following the procedure as described in **5a**, afforded the white product **5f**. Yield: 1.1 g (79%); m. p.: 202–203°C (methanol); R_f: 0.62 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3212, 3235 (NH), 1734 (C=O stretching), 700–750 (C-Cl stretching), 1317 (C=S stretching); ^{1}H-NMR ($CDCl_3$) δ: 5.22 (s, 1H, Ar-OH), 6.71–7.54 (m, 7H, ArH), 7.74 (s, 1H, CONH), 0.43 (s, 1H, NH), 11.82 (s, 1H, NHAr); EI-MS (*m/z*, 100%): 356 [M + 2] (100). Anal. calcd. for $C_{14}H_{11}Cl_2N_3O_2S$: C, 48.50; H, 2.81; N, 10.59. Found: C, 48.50; H, 2.80; N, 10.60.

*Compound **5g***

To a solution of compound **2** (1.5 g, 0.01 mol) in ethanol (50 mL), 2,6-diflurophenyl isothiocyanate (1.7 g, 0.01 mol) was added. The reaction mixture was refluxed for 12 h. Following the procedure as described for **5a**, afforded the white product **5g**. Yield: 1.3 g (87%); m. p.: 212–213°C (methanol); R_f: 0.56 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3217, 3232 (NH), 1738 (C=O stretching), 1313 (C=S stretching); ^{1}H-NMR ($CDCl_3$) δ: 5.35 (s, 1H, Ar-OH), 6.78–7.82 (m, 8H, ArH), 7.68 (s, 1H, CONH), 10.40 (s, 1H, NH), 11.81 (s, 1H, NHAr); EI-MS (*m/z*, 100%): 323 [M + 2] (100). Anal. calcd. for $C_{14}H_{11}F_2N_3O_2S$: C, 52.01; H, 3.39; N, 13.00. Found: C, 52.01; H, 3.43; N, 13.00.

*Compound **5h***

To a solution of compound **2** (1.5 g, 0.01 mol) in ethanol (50 mL), 2,6-dimethylphenyl isothiocyanate (1.6 g, 0.01 mol) was added. The reaction mixture was refluxed for 13 h. Following the procedure as described in **5a** afforded the pale white product **5h**. Yield: 1.0 g (69%); m. p.: 198–199°C (methanol); R_f: 0.66 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3010–3030 (Ar-CH), 3217, 3232 (NH), 1738 (C=O stretching), 1313 (C=S stretching); ^{1}H-NMR ($CDCl_3$) δ: 5.30 (s, 1H, Ar-OH), 2.11 (d, 2H, CH_3), 6.78–7.82 (m, 7H, ArH), 7.66 (s, 1H, CONH), 10.44 (s, 1H, NH), 11.80 (s, 1H, NHAr); EI-MS (*m/z*, 100%): 316 [M + 2] (100%). Anal. $C_{16}H_{17}N_3O_2S$; C, 60.91/60.93; H, 5.39/5.43; N, 13.32/13.32.

*Compound **5i***

To a solution of compound **2** (1.5 g, 0.01 mol) in ethanol (50 mL), 2,6-dimethylphenyl isothiocyanate (1.9 g, 0.01 mol) was added. The reaction mixture was refluxed for 12 h. Following the procedure as described for **5a** afforded the yellowish product **5i**. Yield: 1.12 g (75%); m. p.: 203–204°C (methanol); R_f: 0.57 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3219, 3230 (NH), 1732 (C=O stretching), 1310 (C=S stretching); ^{1}H-NMR ($CDCl_3$) δ: 5.31 (s, 1H, Ar-OH), 3.83 (d, 2H, OCH_3), 6.30–7.82 (m, 7H, ArH), 7.69 (s, 1H, CONH), 10.40 (s, 1H, NH), 11.80 (s, 1H, NHAr); EI-MS (*m/z*, 100%): 347 [M + 2] (100). Anal. calcd. for $C_{16}H_{17}N_3O_4S$: C, 55.31; H, 4.90; N, 12.08. Found: C, 55.32; H, 4.93; N, 12.10.

*General procedure for synthesis of compound **6a–6i***

A mixture of the thiosemicarbazide (0.01 mol), chloroacetic acid (0.01 mol), and sodium acetate (0.2 mol) in ethanol (60 mL) was refluxed for 10 h. The mixture was cooled, diluted with water till turbidity, and left overnight to obtain the product. The product was filtered, dried, and recrystallized using aqueous ethanol.

*Compound **6a***

A mixture of compound **5a** (2.57 g, 0.01 mol), chloroacetic acid (0.01 mol), and sodium acetate (0.2 mol) in ethanol (60 mL) was heated under reflux for 10 h. The mixture was cooled and diluted with enough water to develop turbidity. The mixture was left overnight for complete separation of the product. The separated product was filtered and dried to give the corresponding whitish product **6a**. Yield: 2.21 g (86%); m. p.: 225–226°C (methanol); R_f: 0.55 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3212 (NH stretching), 2986, 2990 ($CH(CH_3)_2$ stretching), 1732 (C=O stretching), 1315 (C=S stretching); ^{1}H-NMR ($CDCl_3$) δ: 1.12 (d, 6H, isopropyl CH_3), 3.96 (m, 1H, isopropyl CH), 5.31 (s, 1H, Ar-OH), 3.01 (s, 2H, thiazolidine CH_2), 7.74 (s, 1H, CONH); EI-MS (*m/z*, 100%): 293 [M + 2] (100). Anal. calcd. for $C_{13}H_{15}N_3O_3S$: C, 53.21; H, 5.15; N, 14.30. Found: C, 53.23; H, 5.15; N, 14.32.

*Compound **6b***

A mixture of compound **5b** (2.67 g, 0.01 mol), chloroacetic acid (0.01 mol) and sodium acetate (0.2 mol) in ethanol (60 mL) was heated under reflux for 10 h. The mixture was cooled and diluted with enough water to develop turbidity. The mixture was left overnight for complete separation of the product. The separated product was filtered and dried to give the corresponding whitish product **6b**. Yield: 2.2 g (85%); m. p.: 206–207°C (methanol); R_f: 0.59 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 1732 (C=O stretching), 3212 (NH stretching); ^{1}H-NMR ($CDCl_3$) δ: 0.95 (t, 3H, butyl CH_3), 1.28 (m, 2H, butyl CH_2), 1.51 (m, 2H, butyl CH_2), 3.53 (m, 2H, butyl CH_2), 3.01 (s, 2H, thiazolidine CH_2), 4.72 (s, 1H, NH), 5.35 (s, 1H, Ar-OH), 7.73 (s, 1H, CONH); EI-MS (*m/z*, 100%): 307 [M + 2] (100). Anal. calcd. for $C_{14}H_{17}N_3O_3S$: C, 53.91; H, 6.35; N, 15.62. Found: C, 53.91; H, 6.41; N, 15.72.

*Compound **6c***

A mixture of compound **5c** (2.8 g, 0.01 mol), chloroacetic acid (0.01 mol), and sodium acetate (0.2 mol) in ethanol (60 mL) was heated under reflux for 10 h. The mixture was cooled and diluted with enough water to develop turbidity. The mixture was left overnight for complete separation of the product. The separated product was filtered and dried to give the corresponding whitish product **6c**. Yield: 2.1 g (84%); m. p.: 256–257°C (methanol); R_f: 0.66 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 1732 (C=O stretching), 3218 (NH stretching); ^{1}H-NMR ($CDCl_3$) δ: 3.04 (s, 2H, thiazolidine CH_2), 4.72 (s, 1H, NH), 5.35 (s, 1H, Ar-OH), 7.73 (s, 1H, CONH), 6.38–7.85 (m, 9H, Ar-H); EI-MS (*m/z*, 100%): 327 [M + 2] (100). Anal. calcd. for $C_{16}H_{13}N_3O_3S$: C, 58.51; H, 4.55; N, 14.52. Found: C, 58.52; H, 4.56; N, 14.62.

*Compound **6d***

A mixture of compound **5d** (3.2 g, 0.01 mol), chloroacetic acid (0.01 mol), and sodium acetate (0.2 mol) in ethanol (60 mL) was heated under reflux for 10 h. The mixture was cooled and diluted with enough water to develop turbidity. The mixture was left overnight for complete separation of the product. The separated

product was filtered and dried to give the corresponding yellowish product **6d**. Yield: 1.6 g (52%); m. p.: 241–242°C (methanol); R_f: 0.56 (acetonitrile/methanol, 1:1), IR (KBr), in cm^{-1}: 3214 (NH stretching), 1731 (C=O stretching), 1555 (NO_2 stretching); ^{1}H -NMR ($CDCl_3$) δ: 3.98 (s, 2H, thiazolidine CH_2), 4.60 (s, 1H, NH), 5.23 (s, 1H, Ar-OH), 7.70 (s, 1H, CONH), 6.38–7.85 (m, 8H, Ar-H); EI-MS (*m*/*z*, 100%): 372 [M + 2] (100). Anal. calcd. for $C_{16}H_{12}N_4O_5S$: C, 50.51; H, 3.55; N, 16.82. Found: C, 50.60; H, 3.64; N, 16.86.

*Compound **6e***

A mixture of compound **5e** (3.0 g, 0.01 mol), chloroacetic acid (0.01 mol), and sodium acetate (0.2 mol) in ethanol (60 mL) was heated under reflux for 10 h. The mixture was cooled and diluted with enough water to develop turbidity. The mixture was left overnight for complete separation of the product. The separated product was filtered and dried to give the corresponding whitish product **6e**. Yield: 2.7 g (89%); m. p.: 223–224°C (methanol); R_f: 0.7 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3220 (NH stretching), 1792 (C=O stretching), 1045 (C-F stretching); ^{1}H-NMR ($CDCl_3$) δ: 3.76 (s, 2H, thiazolidine CH_2), 4.36 (s, 1H, NH), 5.3 (s, 1H, Ar-OH), 8.04 (s, 1H, CONH), 6.38–7.85 (m, 8H, Ar-H); EI-MS (*m*/*z*, 100%): 372 [M + 2] (100). Anal. calcd. for $C_{16}H_{12}FN_3O_3S$: C, 55.05; H, 3.95; N, 13.78. Found: C, 55.07; H, 3.96; N, 13.76.

*Compound **6f***

A mixture of compound **5f** (3.5 g, 0.01 mol), chloroacetic acid (0.01 mol), and sodium acetate (0.2 mol) in ethanol (60 mL) was heated under reflux for 10 h. The mixture was cooled and diluted with enough water to develop turbidity. The mixture was left overnight for complete separation of the product. The separated product was filtered and dried to give the corresponding whitish product **6f**. Yield: 2.6 g (75%); m. p.: 215–216°C (methanol); R_f: 0.56 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3220 (NH stretching), 1743 (C=O stretching); ^{1}H-NMR ($CDCl_3$) δ: 3.76 (s, 2H, thiazolidine CH_2), 4.30 (s, 1H, NH), 5.28 (s, 1H, Ar-OH), 7.82 (s, 1H, CONH), 6.38–7.85 (m, 7H, Ar-H); EI-MS (*m*/*z*, 100%): 396 [M + 2] (100). Anal. calcd. for $C_{16}H_{11}Cl_2N_3O_3S$: C, 47.21; H, 3.11; N, 11.76. Found: C, 47.20; H, 3.11; N, 11.80.

*Compound **6g***

A mixture of compound **5g** (3.2 g, 0.01 mol), chloroacetic acid (0.01 mol), and sodium acetate (0.2 mol) in ethanol (60 mL) was heated under reflux for 10 h. The mixture was cooled and diluted with enough water to develop turbidity. The mixture was left overnight for complete separation of the product. The seprated product was filtered and dried to give the corresponding whitish product **6g**. Yield: 2.4 g (78%); m. p.: 219–220°C (methanol); R_f: 0.56 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3280 (NH stretching), 1773 (C=O stretching); ^{1}H-NMR ($CDCl_3$) δ: 3.06 (s, 2H, thiazolidine CH_2), 4.36 (s, 1H, NH), 5.23 (s, 1H, Ar-OH), 7.82 (s, 1H, CONH), 6.38–7.85 (m, 7H, Ar-H); EI-MS (*m*/*z*, 100%): 363 [M + 2] (100). Anal. calcd. for $C_{16}H_{11}F_2N_3O_3S$: C, 52.01; H, 3.41; N, 13.00. Found: C, 52.01; H, 3.43; N, 13.00.

*Compound **6h***

A mixture of compound **5h** (3.1 g, 0.01 mol), chloroacetic acid (0.01 mol), and sodium acetate (0.2 mol) in ethanol (60 mL) was heated under reflux for 10 h. The mixture was cooled and diluted with enough water to develop turbidity. The mixture was left overnight for complete separation of the product. The separated product was filtered and dried to give the corresponding whitish product **6h**. Yield: 2.6 g (87%); m. p.: 217–218°C (methanol); R_f: 0.61 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3280 (NH stretching), 1773 (C=O stretching); ^{1}H-NMR ($CDCl_3$) δ: 1.47 (d, 3H, CH_3), 3.16 (s, 2H, thiazolidine CH_2), 4.26 (s, 1H, NH), 5.34 (s, 1H, Ar-OH), 7.62 (s, 1H, CONH), 6.48–7.85 (m, 7H, Ar-H); EI-MS (*m*/*z*, 100%): 355 [M + 2] (100). Anal. calcd. for $C_{18}H_{17}N_3O_3S$: C, 60.91; H, 5.43; N, 13.30. Found: C, 60.93; H, 5.43; N, 13.32.

*Compound **6i***

A mixture of compound **5i** (3.4 g, 0.01 mol), chloroacetic acid (0.01 mol), and sodium acetate (0.2 mol) in ethanol (60 mL) was heated under reflux for 10 h. The mixture was cooled and diluted with enough water to develop turbidity. The mixture was left overnight for complete separation of the product. The separated product was filtered and dried to give the corresponding whitish product **6i**. Yield: 2.6 g (84%); m. p.: 203–204°C (methanol); R_f: 0.61 (acetonitrile/methanol, 1:1); IR (KBr), in cm^{-1}: 3280 (NH stretching), 1773 (C=O stretching); ^{1}H-NMR ($CDCl_3$) δ: 3.73 (s, 6H, OCH_3), 3.76 (s, 2H, thiazolidine CH_2), 4.16 (s, 1H, NH), 5.41 (s, 1H, Ar-OH), 7.82 (s, 1H, CONH), 6.48–7.85 (m, 7H, Ar-H); EI-MS (*m*/*z*, 100%): 387 [M + 2] (100). Anal. calcd. for $C_{18}H_{17}N_3O_5S$: C, 55.31; H, 4.90; N, 12.06. Found: C, 55.32; H, 4.93; N, 12.10.

Pharmacological studies

Antihypertensive activity by non-invasive tail-cuff method

The antihypertensive drugs were evaluated by studying their response on elevated blood pressure. A drug that reduces the elevated BP (blood pressure) to normal is said to be antihypertensive drug.

The newly synthesized compounds **4a–4n** (see Scheme 1) and **6a–6i** (Scheme 2) were tested for their antihypertensive activity. A Norwegian strain of inbreed albino rats weighing 200–250 g was used. (The animals were purchased from the National Toxicological Centre, Pune, India.) All rats were housed in a temperature- (25°C) and humidity-controlled room (75 ± 5%) with a 12-h light/dark cycle. Valsertan was used as standard drug.

Induction of hypertension: fructose-induced hypertension

Groups of eight male Wistar rats weighing 210–250 g were used (purchased from National Toxicological Centre, Pune, India). Rats were housed two per cage on a 12-h light and 12-h dark cycle and were allowed free access to standard laboratory diet (Purina rat chow) and drinking fluid. Drinking fluid consisted of a fructose solution (10%). Body weight, food and fluid intake of each rat were measured every week during the treatment. By using the tail-cuff method, systolic blood pressure and pulse rate was measured before and every week during treatment.

Evaluation of hypertension

Since maximum effects on the chosen parameters were achieved after 4–6 weeks, the duration of the treatment could get limited to this time. Statistical analysis was performed using a one-way analysis of variance, followed by the Newman–Keuls test.

The experimental work was carried out in accordance with the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments in Animal (CPCSEA)

The newly synthesized compounds **4a–4n** (Scheme 1) and **6a–6i** (Scheme 2) were used to study antihypertensive activity.

25 groups for NIBP and 7 groups for IBP, consisting of six rats each of Norwegian strain of inbreed albino rats (male), weighing 200–250 g, were used. Valsartan was used as standard drug. All rats were housed in a temperature- and humidity-controlled room with a 12-h light/dark cycle. Antihypertensive activity was tested via two methods viz. non-invasive blood pressure measurement (NIBP), which measures the systolic blood pressure, and invasive blood pressure measurement (IBP), which measures the diastolic blood pressure. Only those compounds which had demonstrated a significant activity in the NIBP were further tested by IBP method.

Non-invasive blood pressure (NIBP) measurement

Indirect (non-invasive) blood pressure was determined with a Power Lab/4SP with ML135 Dual Bio Amp and computerized BP monitor (AD instruments Pvt. Ltd., Australia). This system measures the systolic blood pressure (SBP) by recording the cuff pressure at which the interrupted blood flow returns to the tail.

Hypertension was induced by an increase of fructose in the animals' diet. Rats were trained for the experiment. On the first day of the experiment, the test compounds, *i. e.* **4a–4n** and **6a–6i**, were administered by oral feeding using an oral feeding needle. The test compounds and standard drug valsertan were prepared in carboxymethyl cellulose solution (5%) and given orally (dose 10 mg/kg). One group of animals was treated with standard drug valsartan. One out of the twenty-five groups was treated with vehicle.

The tail pulse was detected by passage of the tail through a narrow tail-cuff sensor attached to the amplifier. BP measurements were started by automatic inflation of the tail-cuff to greater than 200 mmHg and then release of pressure. The results were recorded in form of a graph. The computer was provided with two tracings: as soon as one had started, the other was stopped at the same time. The lower trace channel plotted cuff pressure, which was calibrated at 500 mmHg at full scale. The tracing was sharply raised when applied to the tail cuff and fell off gradually during 15 to 20 s during the test. The upper trace channel monitors the pulse, with fluctuations about the centre line suddenly appearing at the onset of pulsations. The first onset of the pulse was taken as the systolic blood pressure. Initiation of pulse pressure was determined when the baseline amplitude increased in accordance with the set maximal inflated cuff pressures. The maximal inflation was set at 200 mmHg.

Blood pressure recording were considered to be successful when the animal did not move and a clear initial pulse could be seen. Ten tail-cuff measurements were made in a session. The BP for the session was accepted as the average of four BP readings that were within 5 mmHg or the average of ten readings that were within 8 mmHg. BP measurements were done thrice per week for two weeks.

Prior to dosing the animals, the initial graph reading was taken to record the BP before administration of the drug. After 1 h of dosing, recordings were taken as mentioned above. Each compound was evaluated three times in one week, and average of the recordings was noted, accordingly.

The experiment was repeated at two different dose levels (5 and 2.5 mg/kg) for those compounds that showed significant statistical differences between test and control groups. Average readings were calculated by employing the one-way ANOVA method.

Five compounds, *i. e.* **4g**, **4j**, **4l**, **6f**, and **6i**, had shown significant activity by non-invasive blood pressure measurement (tail-cuff method); they were further evaluated for their antihypertensive activity by invasive blood pressure measurement (IBP, direct cannulation of the carotid artery). Antihypertensive activity, carried out by the cannulation method gave the diastolic blood pressure (DBP). The data are presented as means ± S.E.M. A repeated measures analysis of variance was used to obtain the statistical significance between and within groups. The differences were considered statistically significant at $P < 0.05$, and the F value for all compounds was F: ±0.5.

Invasive blood pressure (IBP) measurements

Blood pressure measurements were performed by direct cannulation of the carotid artery. The mean arterial blood pressure (MAP) was measured by cannulation technique employing a Power Lab/4SP with ML135 Dual Bio Amp and computerized BP monitor (AD instruments Pvt. Ltd., Australia).

Animals were anesthetized with sodium pentobarbital (35 mg/kg, *i.p.*), and the left carotid artery was exposed, cannulated, and exteriorized between the scapulas. Blood pressure was measured directly from cannula using transducers attachments of the above instrument. After the animals recovered from surgery, a base-line blood pressure was established. They were dosed orally with the test compounds via a feeding needle. Acute effects were determined by monitoring the blood pressure at 15, 30, 60, 120, 180, and 240 min after oral dosing. Average readings were calculated by employing the ANOVA method.

3D-QSAR study

Data set for analysis

A data set comprising 23 compounds, *i. e.* **4a–4n** and **6a–6i**, were used in the present study. The *in-vivo* biological activity data was reported as IC_{50}. The IC_{50} values were converted to $pIC_{50} = -\log(IC_{50})$.

$$IC_{50} = pIC_{50} = -\log C + \log(\text{it}) \quad (1)$$

where

$$C = \text{molar concentration} = [\text{concentration (mg/mL)} \times 0.001/\text{molecular weight}]$$

$$\log(\text{it}) = \log[\%\text{inhibition}/100 - \%\text{inhibition}].$$

The data set consists of some highly active and inactive compounds, with few compounds in between them. Table 5 represents pIC_{50} values with its actual pIC_{50} values and PHASE-predicted pIC_{50} values. A total of 23 compounds was available with pIC_{50} values, of which 17 compounds were chosen for the training set and six compounds were selected for the test sets based on the Tanimoto similarity coefficient [27].

3D-QSAR

3D-QSAR studies were undertaken by PHASE 3D-QSAR (a high-performance program for ligand-based drug design [25]) model workflow using 23 compounds. The following five steps were performed.

Step I: Preparing ligands

The 3D conversion and minimization was performed using LigPrep (MMFF force field) incorporated in PHASE. A maximum of 100 conformers were generated per structure using a pre-process minimization of 100 steps and post-process minimization of 50 steps. Each minimized conformer was filtered through a relative energy window of 11.4 kcal/mol (50 kJ/mol) and a minimum atom deviation of 2.00 Å.

Step II: Creating pharmacophore sites

The second step in developing a pharmacophore model is to use a set of pharmacophore features to create sites for all the ligands. Each ligand structure is represented by a set of points in 3D space, which coincide with various chemical features that may facilitate noncovalent binding between the ligand and its target receptor. PHASE provides a built-in set of six pharmacophore features, hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic group (H), negatively ionizable (N), positively ionizable (P), and aromatic ring (R). The rules that are applied to map the positions of pharmacophore sites are known as feature definitions, and they are represented internally by a set of SMARTS (Smiles ARbitory Target Specification) patterns. Each pharmacophore feature is defined by a set of chemical structure patterns. All user-defined patterns are specified as SMARTS queries and assigned one of the three possible geometries, which define physical characteristic of the site (i) Point: the site is located on a single atom in the SMARTS query; (ii) Vector: the site is located on a single atom in the SMARTS query, and it will be assigned directionality according to one or more vectors originating from the atom; (iii) Group: the site is located at the centroid of a group of atoms in the SMARTS query. For aromatic rings, the site is assigned directionality defined by a vector that is normal to the plane of the ring. A default setting having acceptor (A), donor (D), hydrophobic (H), negative (N), positive (P), and aromatic ring (R) was used for the creation of pharmacophore sites. No user-defined feature was employed for the present study.

The second step in developing a pharmacophore model was to use a set of pharmacophore features and to create sites for all the ligands. The different sites for pharmacophore were created.

Step III: Finding a common pharmacophore

Active and inactive thresholds of pIC_{50} were 0.2 and 0.3, respectively. The thresholds were applied to the training set for developing the common pharmacophore hypotheses. After applying, default feature definitions to each ligand and common pharmacophores contajning four sites were generated using a terminal box size of 1 Å, and all active compounds match. Any single pharmacophore in the group ultimately becomes a common pharmacophore hypothesis which gives an explanation how ligands bind to the receptor. Common pharmacophores are identified using a tree-based partitioning technique that groups together similar pharmacophores according to their intersite distances, *i. e.*, the distances between pairs of sites in the pharmacophore.

Step IV: Scoring hypotheses

In the score hypotheses step, common pharmacophores are examined, and a scoring procedure is applied to identify the pharmacophore from each surviving *n*-dimensional box that yields the best alignment of the active set ligands. This pharmacophore provides a hypothesis to explain how the active molecules bind to the receptor. The scoring procedure provides a ranking of the different hypotheses, which allows making rational choices about which hypotheses are most appropriate for further investigation. Scoring with respect to actives was conducted using default parameters for site, vector, and volume terms. Ligand activity, expressed as $-\log_{10}(IC_{50})$, was incorporated into the score with a weight of 1.0, and relative conformational energy (kJ/mol) was included with a weight of 0.01. Hypotheses that emerged from this process were subsequently scored with respect to inactives, using a weight of 1.0. The inactive molecules were scored to observe the alignment of these molecules with respect to the pharmacophore hypothesis to enable making a decision on the selection of the hypothesis. Larger is the difference between the scores of active and inactives, better is the hypothesis at distinguishing the actives from inactives. Their results are summarized in Table 6.

Step V: Building QSAR model

Atom-based 3D-QSAR models were generated for the hypotheses using the 17 compound in the training set with three partial least square (PLS) factors and a grid spacing of 1 Å. The 3D-QSAR results were visualized using 3D plots of crucial pharmacophore regions (Fig. 3). As compound **4j** showed good activity, the common pharmacophores were generated for the best PHASE hypothesis with this compound (Fig. 3a). PHASE 3D-plots of crucial pharmacophore regions based on the hypothesis generated were displayed with compound **4j**. Positive coefficient-favored areas (contributing for increase in activity) are represented by gray cubes. Negative coefficient-favored areas (contributing for decrease in activity) are represented by black cubes. The visual representations are covered in Figs. 3b to 3d. The summary of PHASE 3D-QSAR statistical analysis is given in Table 7.

PHASE-QSAR statistics and model validation

The PHASE descriptors served as independent variables and activity values as dependent variables in deducing 3D-QSAR models by PLS regression analysis method. PHASE-QSAR models do not use internal cross-validation techniques but rather uses distinct training and test sets. The true external test sets assists in the validation of the 3D-QSAR model and helps in the generation of validation parameters like q^2 and r_p values. The predictive ability of each analysis was determined from a test of six ligands that were not included in the training set. The test set molecules were aligned and their activities were predicted by each PLS analysis. The statistical results are summarized in Table 7.

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