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ORIGINAL ARTICLE

Synthesis and antimicrobial evaluation of novel 1,3,4-thiadiazole derivatives of 2-(4-formyl-2-methoxyphenoxy) acetic acid

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Abstract A series of 1,3,4-thiadiazole derivatives of 2-(4-formyl-2-methoxyphenoxy) acetic acid (**6a–s**) were synthesized by cyclization of carboxylic acid group of 2-(2-methoxy-4-(3-oxo-3-substituted phenylprop-1-enyl)phenoxy) acetic acid (**4a–s**) with thiosemicarbazide in the presence of $POCl_3$ or PPA. The structures of the compounds were confirmed by IR, ^1H NMR and mass analysis. All the compounds have been evaluated *in vitro* for their antimicrobial activities against several strains of microbes and show significant activity.

1. Introduction

The treatment of infectious disease caused by bacteria, fungi and viruses still remains an important and challenging problem because of a combination factors including newly emerging infectious diseases and increasing number of multi-drug resistant gram-positive pathogens (Tenover and McDonald, 2005; Pfeltz and Wilkinson, 2004; Roberts, 2004; Dessen et al., 2001), such as methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant *Enterrococci* (VRE), compounded problems in the therapeutics (Babu et al., 2008; Dalhoff, 1994). Thus it is still necessary to search for new antimicrobial agents.

Five membered aromatic systems having three hetero atoms at symmetrical position have interesting physiological properties (Hetzhein and Mockel, 1996; Sandstrom, 1968). During recent years there has been intense investigation of different classes of thiadiazole compounds, many of which known to

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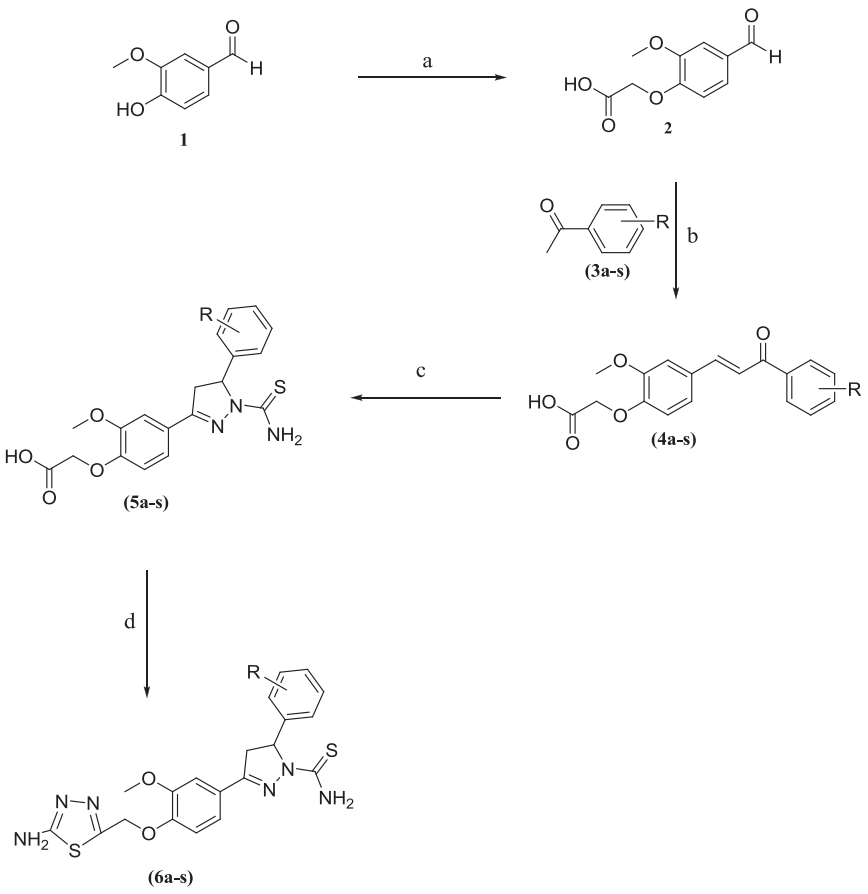

R= **a)** H; **b)** 2-OCH_3; **c)** 2,4-di-Cl; **d)** 3-NH_2; **e)** 3-NO_2; **f)** 4-OCH_3; **g)** 4-F; **h)** 4-NO_2; **i)** 4-Br; **j)** 4-CH_3; **k)** 3-OH; **l)** 2-OH; **m)** 4-Cl; **n)** 2-NH_2; **o)** 2,4-diOH; **p)** 4-NH_2; **q)** 2-Cl; **r)** 4-OH; **s)** 3-CH_3

Scheme 1 Reagents: (a) chloroacetic acid, NaOH, HCl; (b) EtOH, KOH, petroleum ether; (c) thiosemicarbazide, glacial acetic acid; (d) thiosemicarbazide, PPA or $POCl_3$.

possess interesting biological properties such as antimicrobial (Demirbas et al., 2009; Kadi et al., 2007; Bekhit and Abdel-Aziem, 2004), anti-inflammatory (Mullican et al., 1993; Song et al., 1999; Mathew et al., 2007), anticonvulsants (Chapleo et al.,1986, 1988), antioxidant (Cressier et al., 2009), anticancer (Matysiak et al., 2006; Chou et al., 2003; Radi et al., 2008) and antifungal (Swamy et al., 2006) activities. The activity of 1,3,4-thiadiazoles is possibly due to the presence of the =N–C–S moiety (Bauer et al., 1966). In view of these facts, we have synthesized several new 1,3,4-thiadiazole derivatives of 2-(4-formyl-2-methoxyphenoxy) acetic acid moiety in order to study their biodynamic behavior. The present study reports the synthesis of 3-(4-((5-amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-(substituted)-4,5-dihydro-1*H*-pyrazole-1 carbothioamide (**6a**–**s**) by appropriate methods and their evaluation for antibacterial and antifungal potentials.

2. Chemistry

The synthetic route of compounds (**6a**–**s**) is shown in Scheme 1. 2-(4-Formyl-2-methoxyphenoxy) acetic acid (**2**) was prepared by reacting vanillin with chloroacetic acid in the presence of sodium hydroxide. Various chalcone derivatives (**4a**–**s**) were synthesized by treating (**2**) with different derivatives of acetophenone (**3a**–**s**). Compounds (**5a**–**s**) were obtained by refluxing (**4a**–**k**) and thiosemicarbazide in the presence of glacial acetic acid and ethanol. 1,3,4-Thiadiazole derivatives (**6a**–**s**) were obtained by cyclization of (**5a**–**s**) by treating with thiosemicarbazide and $POCl_3$ or PPA. The physical data of all the synthesized compounds is shown in Table 1.

3. Biological activity

All the synthesized 1,3,4-thiadiazole derivatives (**6a**–**s**) have been screened for both antibacterial and antifungal activities using cup-plate agar diffusion method by measuring zone of inhibition in mm. Eight different bacterial cultures *S. aureus, Salmonella enterica, Vibrio cholera, Bacillus subtilis, Proteus mirabili, Escherichia coli* V517, *Mycobacterium smegmatics, Pseudomonas aeruginosa* in nutrient agar medium, and one fungal culture *Candida albicans* in sabouraud's dextrose agar medium (Holla et al., 2002) were used. The results were com-

pared with positive control, the standard drug ampicillin (50 μg/ml) for bacteria and amphotericin B (50 μg/ml) for fungi and negative control, the DMSO poured disk. These sterilized agar media were poured into petri-dishes and allowed to solidify. On the surface of the media microbial suspensions were spread with the help of sterilized triangular loop. A stainless steel cylinder of 8 mm diameter (pre-sterilized) was used to bore cavities. All the synthesized compounds (50 μg/ml) were placed serially in the cavities with the help of micropipette and allowed to diffuse for 1.0 h. DMSO was used as a solvent for all the compounds, and as a control. These plates were incubated at 37 °C for 24 h and 28 °C for 48 h, for the antibacterial and antifungal activities, respectively. The zone of inhibition was observed around the cup after respective incubation and was measured and percent inhibition of the compounds was calculated.

4. Results and discussion

The structures of synthesized compounds were established on the basis of their spectral data. Spectral data of compounds were in full agreement with proposed structures. The formation of 1,3,4-thiadiazoles (**6a**–**s**) was supported by the presence of N–H band in the IR spectra and absence of carbonyl stretching band of the carboxylic acid function. In general, infra red spectra (IR) revealed a bilobe of NH_2 stretch at ~3100, 3300, and C=N, C–N and C=S peak at ~1530, 1328, and 1126 cm^{-1}, respectively. In the nuclear magnetic resonance spectra (^{1}H NMR) the signals of the respective protons of the prepared titled compounds were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The spectra showed a singlet at δ ~5.2 ppm corresponding to OCH_2 group; doublet at δ ~4.0 ppm corresponding to C4 methylene group; singlet at δ ~3.8 ppm corresponding to methoxy group; singlet at δ ~3.2 ppm corresponding to C5 CH group; singlet at δ ~5.8 ppm corresponding to NH_2 and multiplet at δ ~6.5–8.2 ppm for aromatic proton; singlet at δ ~8.5 ppm corresponding to another NH_2 group.

The 1,3,4-thiadiazole derivative **6h** showed activity against all the strains. It showed maximum activity (97%) against *S. enterica* (95%), against *V. cholera* and (87.9%) inhibition of *E. coli* V517 when compared with standard drug ampicillin. Compound **6e** showed (93.2% and 89.5% inhibition) against *S. enterica* and *V. cholera*, respectively. Compound **6k** showed maximum inhibition (87.1%) against *S. aureus*. Compound **6o** was found to be active (96.5% inhibition) against *E. coli* V517 and **6p** showed (90.2% inhibition) against *P. aeruginosa*. Rest of all the 1,3,4-thiadiazole derivatives showed moderate to good antibacterial activity. The 1,3,4-thiadiazole derivative **6h** showed maximum inhibition (87.8%) whereas, compound **6e** showed (83.3%) inhibition against fungal strain *C. albicans*. Rest of all the 1,3,4-thiadiazole derivatives showed moderate to low antifungal activity. The results are presented in Table 2.

5. Experimental

Chemicals were purchased from Merck India, Spectrochem and Sigma–Aldrich etc. Most of the solvents and chemicals used were of LR grade. The purity of the compounds was confirmed by thin layer chromatography using precoated TLC plates and solvent systems of benzene–acetone (9:1), (8:2); T–E–F (5:4:1), and chloroform–methanol (9:1), (9.5:0.5). The spots were visualized under ultraviolet lamp. Melting points were determined in one end open capillary tubes on a liquid paraffin bath and are uncorrected. Infrared (IR) and ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds on Perkin Elmer IR (ν_{max} in cm^{-1}) spectrophotometer in KBr pellets and Bruker model avance II (400 MHz, ^{1}H NMR) instrument, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard.

Table 1 Physical property data of compounds (**6a**–**s**).

Compound	R	Molecular formula	Yield (%)	Melting point (°C)
6a	H	$C_{20}H_{20}N_6O_2S_2$	42	262–267
6b	2-OCH_3	$C_{21}H_{22}N_6O_3S_2$	44	274–278
6c	2,4-di-Cl	$C_{20}H_{18}Cl_2N_6O_2S_2$	36	232–238
6d	3-NH_2	$C_{20}H_{21}N_7O_2S_2$	26	252–256
6e	3-NO_2	$C_{20}H_{19}N_7O_4S_2$	36	298–303
6f	4-OCH_3	$C_{21}H_{22}N_6O_3S_2$	42	268–272
6g	4-F	$C_{20}H_{19}FN_6O_2S_2$	20	244–247
6h	4-NO_2	$C_{20}H_{19}N_7O_4S_2$	44	302–306
6i	4-Br	$C_{20}H_{19}BrN_6O_2S_2$	66	274–279
6j	4-CH_3	$C_{21}H_{22}N_6O_2S_2$	62	282–286
6k	3-OH	$C_{20}H_{20}N_6O_3S_2$	66	312–317
6l	2-OH	$C_{20}H_{20}N_6O_3S_2$	68	258–262
6m	4-Cl	$C_{20}H_{19}ClN_6O_2S_2$	65	228–232
6n	2-NH_2	$C_{20}H_{21}N_7O_2S_2$	59	264–268
6o	2,4-diOH	$C_{20}H_{20}N_6O_4S_2$	67	306–310
6p	4-NH_2	$C_{20}H_{21}N_7O_2S_2$	70	222–226
6q	2-Cl	$C_{20}H_{19}ClN_6O_2S_2$	65	254–258
6r	4-OH	$C_{20}H_{20}N_6O_3S_2$	69	288–292
6s	3-CH_3	$C_{21}H_{22}N_6O_2S_2$	64	272–276

Table 2 Antimicrobial activity data for compounds (**6a–s**).

Compound	% Inhibition								
	S. aureus	*S. enterica*	*V. cholera*	*B. subtilis*	*P. mirabilis*	*E. coli* V517	*M. smegmatics*	*P. aeruginosa*	*C. albicans*
6a	–	–	–	49.6	–	62.9	33.7	44.7	–
6b	–	76.8	38.2	54.7	66.6	77.0	63.9	69.4	–
6c	75	85.0	78.3	–	66.6	82.7	–	–	70.5
6d	–	68.6	–	59.8	–	–	58.1	62.6	48.0
6e	78.5	93.2	89.5	77.3	80.0	84.4	75.5	74.6	83.3
6f	–	78.3	44.4	59.8	70.0	80.1	67.4	74.6	–
6g	75.7	76.8	83.3	80.2	77.3	87.9	72.0	83.5	77.5
6h	80.7	97.0	95.0	81.7	87.3	8.9	81.3	82.8	87.8
6i	–	69.4	52.4	49.6	46.6	62.9	–	–	53.8
6j	–	73.1	46.9	59.1	52.0	79.3	58.1	58.2	–
6k	87.1	79.8	–	–	–	64.6	–	–	55.1
6l	77.1	73.8	–	–	59.3	75.8	–	–	62.8
6m	80.0	79.1	71.4	–	58	85.3	80.2	73.1	72.4
6n	–	70.8	–	67.8	–	88.7	70.3	75.3	62.8
6o	84.2	76.8	–	78.8	70.6	96.5	79.6	86.5	66
6p	–	86.5	–	82.4	–	83.6	83.1	90.2	51.2
6q	81.4	85.8	79	70	66	73.2	74.4	60.4	69.8
6r	76.4	81.3	–	85.4	–	–	65.6	55.9	63.4
6s	–	70.8	54.9	70.8	52	75	73.2	81.3	–
Stand.	100	100	100	100	100	100	100	100	100

(–): No zone of inhibition.
Std.: Standard (ampicillin for bacteria and amphotericin B for fungi).

*5.1. Synthesis of 2-(4-formyl-2-methoxyphenoxy) acetic acid (**2**)*

Compound (**2**) was prepared by the procedure given in the literature (Zubrys and Stebenmann, 1954).

*5.2. Synthesis of 2-{4-[3-(substituted)-3-oxo-1-propenyl]-2-methoxyphenoxy} acetic acid (**4a–k**) and 2-{4-[1-amino (thioxo) methyl-5-(substituted phenyl)-4,5-dihydro-1H-3-pyrazolyl]-2-methoxyphenoxy} acetic acid (**5a–k**)*

Compounds (**4a–k**) and (**5a–k**) were prepared by the procedure given in the literature (Mohammad and Mohammad, 2007).

*5.3. General procedure for the synthesis of 3-(4-((5-amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-(substituted)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6a–k**)*

A mixture of (**5a–k**) (0.05 mol), thiosemicarbazide (0.05 mol) and $POCl_3$ (13 ml) was heated at 75 °C for 0.75 h. After cooling down to room temperature, water was added. The reaction mixture was refluxed for 4 h. After cooling, the mixture was basified to pH 8 by the drop-wise addition of 50% NaOH solution under stirring. The precipitate was filtered and recrystallized from ethanol.

*5.3.1. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6a**)*

Yield 42%; mp: 262–267 °C; IR (KBr, cm^{-1}): 3327.40, 3290.63 (N–H stretch), 3010.29 (Ar C–H stretch), 2919.01 (C–H stretch), 1570.29 (Ar C=C stretch); ^{1}H NMR (DMSO-d_6): 9.19 (s, 2H, NH_2), 6.72–7.94 (m, 8H, Ar-H), 5.48 (s, 2H, NH_2), 5.12 (s, 2H, OCH_2), 4.00 (d, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.41 (s, 1H, CH), MS (*m*/*z* %): 441.08 $[M+1]^+$.

*5.3.2. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-(2-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6b**)*

Yield 44%; mp: 274–278 °C; IR (KBr, cm^{-1}): 3157.02, 3111.20 (N–H stretch), 3041.76 (Ar C–H stretch), 2986.18 (C–H stretch), 1570.41 (Ar C=C stretch); ^{1}H NMR (DMSO-d_6): 9.26 (s, 2H, NH_2), 7.26–8.26 (m, 7H, Ar-H), 5.78 (s, 2H, NH_2), 5.20 (s, 2H, OCH_2), 4.23 (d, 2H, CH_2), 3.82 (s, 6H, OCH_3), 3.53 (s, 1H, CH); MS (*m*/*z* %): 471.18 $[M+1]^+$.

*5.3.3. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6c**)*

Yield 36%; mp: 232–238 °C; IR (KBr, cm^{-1}): 3349.34, 3329.20 (N–H stretch), 3086.49 (Ar C–H stretch), 2998.26 (C–H stretch), 1559.31 (Ar C=C stretch), ^{1}H NMR (DMSO-d_6): 8.92 (s, 2H, NH_2), 6.75–7.98 (m, 6H, Ar-H), 5.89 (s, 2H, NH_2), 5.29 (s, 2H, OCH_2), 4.24 (d, 2H, CH_2), 3.83 (s, 3H, OCH_3), 3.63 (s, 1H, CH); MS (*m*/*z* %): 508. 09 $[M^+]$, 509.11 $[M+1]^+$.

*5.3.4. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-(3-aminophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6d**)*

Yield 26%; mp: 252–256 °C; IR (KBr, cm^{-1}): 3263.24, 3230.38 (N–H stretch), 3018.76 (Ar C–H stretch), 2986.18 (C–H stretch), 1598.83 (Ar C=C stretch); ^{1}H NMR (DMSO-d_6): 12.6 (s, 2H, NH_2), 10.1 (s, 2H, NH_2), 6.72–8.44 (m, 7H, Ar-H), 5.38 (s, 2H, NH_2), 5.15 (s, 2H, OCH_2), 4.15 (d, 2H,

CH_2), 3.80 (s, 3H, OCH_3), 3.26 (s, 1H, CH); MS (*m*/*z* %): 455.15 [M^+].

*5.3.5. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-(3-nitrophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6e**)*

Yield 36%; mp: 298–303 °C; IR (KBr, cm^{-1}): 3129.46, 3116.16 (N–H stretch), 3014.18 (Ar C–H stretch), 2912.64 (C–H stretch), 1545.45 (Ar C═C stretch); ^{1}H NMR (DMSO-d_6): 8.59 (s, 2H, NH_2), 6.70–7.51 (m, 7H, Ar-H), 5.42 (s, 2H, NH_2), 5.07 (s, 2H, OCH_2), 3.95 (d, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.50 (s, 1H, CH); MS (*m*/*z* %): 485.10 [M^+].

*5.3.6. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6f**)*

Yield 42%; mp: 268–272 °C; IR (KBr, cm^{-1}): 3187.53, 3157.32 (N–H stretch), 3027.35 (Ar C–H stretch), 2995.45 (C–H stretch), 1562.80 (Ar C═C stretch); ^{1}H NMR (DMSO-d_6): 9.24 (s, 2H, NH_2), 7.24–8.22 (m, 7H, Ar-H), 5.89 (s, 2H, NH_2), 5.29 (s, 2H, OCH_2), 4.14 (d, 2H, CH_2), 3.82 (s, 6H, OCH_3), 3.68 (s, 1H, CH); MS (*m*/*z* %): 471.08 [$M+1]^+$.

*5.3.7. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6g**)*

Yield 20%; mp: 244–247 °C; IR (KBr, cm^{-1}): 3363.09, 3352.25 (N–H stretch), 3130.92 (Ar C–H stretch), 2969.22 (C–H stretch), 1593.69 (Ar C═C stretch); ^{1}H NMR (DMSO-d_6): 9.48 (s, 2H, NH_2), 6.60–7.79 (m, 7H, Ar-H), 5.83 (s, 2H, NH_2), 5.27 (s, 2H, OCH_2), 4.30 (d, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.56 (s, 1H, CH); MS (*m*/*z* %): 458.12 [M^+].

*5.3.8. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6h**)*

Yield 44%; mp: 302–306 °C; IR (KBr, cm^{-1}): 3299.55, 3257.54 (N–H stretch), 3051.18 (Ar C–H stretch), 2992.21 (C–H stretch), 1590.21 (Ar C═C stretch); ^{1}H NMR (DMSO-d_6): 8.28 (s, 2H, NH_2), 6.69–7.94 (m, 7H, Ar-H), 5.83 (s, 2H, NH_2). 5.27 (s, 2H, OCH_2), 4.24 (d, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.67 (s, 1H, CH); MS (*m*/*z* %): 485.04 [M^+].

*5.3.9. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-(4-bromophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6i**)*

Yield 66%; mp: 274–279 °C; IR (KBr, cm^{-1}): 3186.70, 3157.40 (N–H stretch), 3025.65 (Ar C–H stretch), 2963.90 (C–H stretch), 1570.29 (Ar C = C stretch); ^{1}H NMR (DMSO-d_6): 9.26 (s, 2H, NH_2), 6.69–8.32 (m, 7H, Ar-H), 5.67 (s, 2H, NH_2), 5.27 (s, 2H, OCH_2), 4.27 (d, 2H, CH_2), 3.86 (s, 3H, OCH_3), 3.56 (s, 1H, CH); MS (*m*/*z* %): 518.08 [M^+], 519.01 [$M+1]^+$.

*5.3.10. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-p-tolyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6j**)*

Yield 62%; mp: 282–286 °C; IR (KBr, cm^{-1}): 3195.20, 3169.97 (N–H stretch), 3063.47 (Ar C–H stretch), 2956.65 (CH_3 stretch), 1585.97 (Ar C═C stretch); ^{1}H NMR (DMSO-d_6): 8.59 (s, 2H, NH_2), 6.72–7.94 (m, 7H, Ar-H), 5.48 (s, 2H, NH_2), 5.12 (s, 2H, OCH_2), 4.08 (d, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.41 (s, 1H, CH), 2.55 (s, 3H, CH_3); MS (*m*/*z* %): 454.21 [M^+].

*5.3.11. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-(3-hydroxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6k**)*

Yield 66%; mp: 312–317 °C; IR (KBr, cm^{-1}): 3612.26 (O–H stretch), 3166.10, 3152.56 (N–H stretch), 3020.71 (Ar C–H stretch), 2998.16 (C–H stretch), 1594.25(Ar C═C stretch); ^{1}H NMR (DMSO-d_6): 10.71 (s, 1H, OH), 9.97 (s, 2H, NH_2), 7.61–8.41 (m, 7H, Ar-H), 5.48 (s, 2H, NH_2), 4.02 (s, 2H, OCH_2), 4.05 (d, 2H, CH_2), 3.80 (s, 3H, OCH_3), 3.50 (s, 1H, CH); MS (*m*/*z* %): 456.14 [M^+].

*5.3.12. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-(2-hydroxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6l**)*

Yield 68%; mp: 258–262 °C; IR (KBr, cm^{-1}): 3626.26 (O–H stretch), 3157.20, 3145.56 (N–H stretch), 3059.21 (Ar C–H stretch), 2967.66 (C–H stretch), 1568.35 (Ar C═C stretch); ^{1}H NMR (DMSO-*d6*): 10.24 (s, 1H, OH), 9.53 (s, 2H, NH_2), 7.82–8.66 (m, 7H, Ar-H), 5.50 (s, 2H, NH_2), 4.46 (s, 2H, OCH_2), 4.35 (d, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.32 (s, 1H, CH); MS (*m*/*z* %): 456.09 [M^+].

*5.3.13. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6m**)*

Yield 65%; mp: 228–232 °C; IR (KBr, cm^{-1}): 3134.40, 3167.96 (N–H stretch), 3040.85 (Ar C–H stretch), 2967.07 (C–H stretch), 1559.59 (Ar C═C stretch); ^{1}H NMR (DMSO-*d6*): 9.32 (s, 2H, NH_2), 7.34–8.24 (m, 7H, Ar-H), 5.26 (s, 2H, NH_2), 4.35 (s, 2H, OCH_2), 4.03 (d, 2H, CH_2), 3.86 (s, 3H, OCH_3), 3.20 (s, 1H, CH); MS (*m*/*z* %): 475.02 [$M+1]^+$.

*5.3.14. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-(2-aminophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6n**)*

Yield 59%; mp: 264–268 °C; IR (KBr, cm^{-1}): IR (KBr, cm^{-1}): 3283.34, 3270.08 (N–H stretch), 3027.16 (Ar C–H stretch), 2976.08 (C–H stretch), 1578.93 (Ar C═C stretch); ^{1}H NMR (DMSO-d_6): 11.3 (s, 2H, NH_2), 10.8 (s, 2H, NH_2), 6.74–8.41 (m, 7H, Ar-H), 5.76 (s, 2H, NH_2), 5.36 (s, 2H, OCH_2), 4.20 (d, 2H, CH_2), 3.87 (s, 3H, OCH_3), 3.24 (s, 1H, CH); MS (*m*/*z* %): 455.19 [M^+].

*5.3.15. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-(2,4-dihydroxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6o**)*

Yield 67%; mp: 306–310 °C; IR (KBr, cm^{-1}): 3662.26 (O–H stretch), 3156.50, 3146.76 (N–H stretch), 3020.71 (Ar C–H stretch), 2978.76 (C–H stretch), 1598.95 (Ar C═C stretch); ^{1}H NMR (DMSO-d_6): 11.01 (s, 1H, OH), 10.35 (s, 1H, OH), 9.78 (s, 2H, NH_2), 7.78–8.45 (m, 7H, Ar-H), 5.67 (s, 2H, NH_2), 4.37 (s, 2H, OCH_2), 4.10 (d, 2H, CH_2), 3.87 (s, 3H, OCH_3), 3.08 (s, 1H, CH); MS (*m*/*z* %): 472.22 [M^+].

*5.3.16. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-(4-aminophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6p**)*

Yield 70%; mp: 222–226 °C; IR (KBr, cm^{-1}): 3157.80, 3146.06 (N–H stretch), 3056.51 (Ar C–H stretch), 2989.61 (C–H

stretch), 1549.52 (Ar C=C stretch); ^{1}H NMR (DMSO-d_6): 10.2 (s, 2H, NH_2), 9.87 (s, 2H, NH_2), 7.98–8.89 (m, 7H, Ar-H), 5.87 (s, 2H, NH_2), 4.67 (s, 2H, OCH_2), 4.31 (d, 2H, CH_2), 3.83 (s, 3H, OCH_3), 3.56 (s, 1H, CH); MS (*m*/*z* %): 455.22 [M^+].

*5.3.17. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6q**)*

Yield 65%; mp: 254–258 °C; IR (KBr, cm^{-1}): 3189.20, 3179.36 (N–H stretch), 3067.17 (Ar C–H stretch), 2978.87 (C–H stretch), 1578.52 (Ar C=C stretch); ^{1}H NMR (DMSO-d_6): 8.98 (s, 2H, NH_2), 7.21–8.81 (m, 7H, Ar-H), 5.87 (s, 2H, NH_2), 4.85 (s, 2H, OCH_2), 4.29 (d, 2H, CH_2), 3.83 (s, 3H, OCH_3), 3.78 (s, 1H, CH); MS (*m*/*z* %): 475.23 [$M+1$]$^+$.

*5.3.18. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6r**)*

Yield 69%; mp: 288–292 °C; IR (KBr, cm^{-1}): 3645.54 (O–H stretch), 3177.10, 3168.06 (N–H stretch), 3045.91 (Ar C–H stretch), 2930.09 (C–H stretch), 1578.76 (Ar C=C stretch); ^{1}H NMR (DMSO-d_6): 10.98 (s, 1H, OH), 9.87 (s, 2H, NH_2), 7.89–8.78 (m, 7H, Ar-H), 5.89 (s, 2H, NH_2), 4.87 (s, 2H, OCH_2), 4.60 (d, 2H, CH_2), 3.80 (s, 3H, OCH_3), 3.64 (s, 1H, CH); MS (*m*/*z* %): 456.14 [M^+].

*5.3.19. 3-(4-((5-Amino-1,3,4-thiadiazol-2-yl)methoxy)-3-methoxyphenyl)-5-m-tolyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (**6s**)*

Yield 64%; mp: 272–276 °C; IR (KBr, cm^{-1}): 3167.90, 3187.16 (N–H stretch), 3034.91 (Ar C–H stretch), 2999.16 (C–H stretch), 1580.75 (Ar C=C stretch); ^{1}H NMR (DMSO-d_6): 8.97 (s, 2H, NH_2), 7.91–8.71 (m, 7H, Ar-H), 5.49 (s, 2H, NH_2), 4.30 (s, 2H, OCH_2), 4.16 (d, 2H, CH_2), 3.89 (s, 3H, OCH_3), 3.02 (s, 1H, CH), 2.67 (s, 3H, CH_3); MS (*m*/*z* %): 454.02 [M^+].

6. Conclusion

A total of 19 compounds were synthesized and screened for their antibacterial activity against *S. aureus*, *S. enterica*, *V. cholera*, *B. subtilis*, *P. mirabili*, *E.* coli V517, *M. smegmatics*, *P.* aeruginosa and antifungal activity against *C. albicans*. The % inhibition of all the compounds was determined by observing the zones of inhibition formed around the cup after 24 h of incubation for antibacterial and 48 h for antifungal activities. Among the tested compounds **6h**, **6e**, **6k**, **6o**, **6p** and **6h**, **6e** possess significant antibacterial and antifungal activities, respectively while rest of all the 1,3,4-thiadiazole derivatives showed moderate antimicrobial activity as compared to standards.

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