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Original article

Synthesis, characterisation and biological activity of novel 4-thiazolidinones, 1,3,4-oxadiazoles and some related compounds

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Abstract

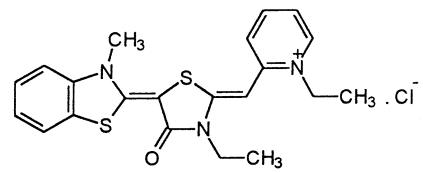
Two novel series of 4-thiazolidinone derivatives namely 2-substituted-3-{[4-(4-methoxybenzoylamino)benzoyl]amino}-4-thiazolidinones (**7a**–**e**) and 2-[4-(4-methoxybenzoylamino)benzoylhydrazono]-3-alkyl-4-thiazolidinones (**5a**–**c**) together with 2-[4-(4-methoxybenzoylamino)phenyl]-5-(substituted phenyl)amino-1,3,4-oxadiazoles (**6a**–**c**) have been synthesised as title compounds. N^1-[4-(4-methoxybenzoylamino)benzoyl]-N^2-substituted methylene hydrazines (**3a**–**e**) and 1-[4-(4-methoxybenzoylamino)benzoyl]-4-substituted phenyl thiosemicarbazides (**4a**–**f**) were also prepared and used as intermediate to give the title compounds. All synthesised compounds were screened for their antimycobacterial activity against *Mycobacterium tuberculosis H37Rv* and antimicrobial activities against various bacteria and fungi. Compounds **7a** and **7b** were found as the most active derivatives demonstrating 90 and 98% inhibition of mycobacterial growth of *M. tuberculosis H37Rv* in the primary screen at 6.25 μg mL^{-1}, respectively. However, level II assay revealed that the MIC values were not less than 6.25 μg mL^{-1}. None of the compounds showed significant antimicrobial activity against the microorganisms used whereas **3a** and **7a** inhibited the growth of several bacteria and fungi. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: 4-thiazolidinone; 1,3,4-oxadiazole; hydrazide–hydrazone; thiosemicarbazide; antimycobacterial activity; BACTEC 460 radiometric system

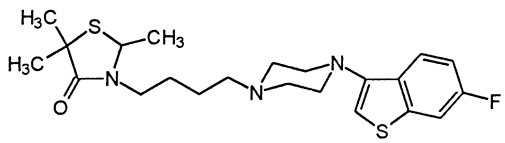
1. Introduction

4-Thiazolidinone derivatives have been demonstrated to possess antibacterial [1–6], antifungal [2,7–10], anticonvulsant [11–14], anticancer [15] and antituberculosis [16,17] activities. Compounds MKT 077 [18] and HP-236 [19] have been reported as a registered antitumour and antipsychotic agents, respectively (Fig. 1).

4-Thiazolidinones have been reported as novel inhibitors of the bacterial enzyme Mur B which was a precursor acting during the biosynthesis of peptidoglycan [20]. Moreover, anticonvulsant [21,22], antibacterial [23] and antifungal [24] properties of several N^1-[4-(4-methoxybenzoylamino)benzoyl]-N^2-substituted methylene hydrazines and 1-[4-(4-methoxybenzoylamino)-benzoyl]-4-alkyl–aryl thiosemicarbazides were described.



[MKT 077]



[HP-236]

Fig. 1.

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In view of the above observations, the synthesis of novel 4-thiazolidinone derivatives starting from either hydrazide–hydrazones or aroylthiosemicarbazides were aimed at investigating biological activities of these compounds.

2. Chemistry

Synthetic route to compounds **3a**–**e**, **4a**–**f**, **5a**–**c**, **6a**–**c**, **7a**–**e** are shown in Fig. 2. Ethyl 4-(4-methoxybenzoylamino)benzoate (**1**) and 4-(4-methoxybenzoylamino)benzoyl hydrazine (**2**) were prepared according to the method described by Rollas et al. [24]. Condensation of **2** with appropriate aldehydes yielded the corresponding hydrazide–hydrazones **3a**–**e**, which on condensation with mercaptoacetic acid afforded 4-thiazolidinones **7a**–**e**. Acylthiosemicarbazides **4a**–**f** were prepared by the addition of 4-(4-methoxybenzoylamino)benzoyl hydrazine (**2**) to various isothiocyanates. Of these compounds, **4a** (R = $-CH_3$), **4b** (R = $-C_2H_5$), **4c** (R = $-CH_2-CH=CH_2$) and **4d** (R = $-C_6H_5$) have been reported elsewhere [24] whereas compounds **4e** and **4f** were synthesised as novel compounds in the present study. Compounds **4a**–**f** were reacted with ethyl bromoacetate in the presence of anhydrous sodium acetate to give the desired 4-thiazolidinones (**5a**–**c**). However, formation of desired 4-thiazolidinones from (**4d**–**f**) failed and instead 1,3,4-oxadiazoles (**6a**–**c**) were obtained (Fig. 2). Structures of these compounds were characterised using UV, ^{1}H-NMR, ^{13}C-NMR and EI-mass spectral data. Physical and spectral data are given Tables 1 and 2.

In the UV spectra of arylthiosemicarbazide derivatives **4e** and **4f**, which were obtained from the reaction of the hydrazide **2** with 4-methyl–methoxyphenyl isothiocyanates, two absorption maxima were shown at 205–206 and 292 nm [25]. The ^{1}H-NMR spectra of compounds **4e** and **4f** displayed the –CON*H*NH–, –CONHN*H*CS– and –CSN*H*C_6H_4– resonances of thiosemicarbazides at 10.42–10.41, 9.74–9.68 and 9.62–9.57 ppm as singlets, respectively [26]. The structures of **4a**–**d** were described previously [24]. The UV spectra of hydrazide–hydrazone derivatives **3a**–**e**, which were obtained by the action of appropriate aldehydes on the hydrazide **2**, exhibited characteristic K bands arising from chromophoric –C=N– group at 312–365 nm [27]. Of these series, only compound **3a** containing a nitro function, showed batochromic shift. In the ^{1}H-NMR spectra of compounds **3a**–**e**, the signals representing the azomethine protons appeared at 8.33–8.46 ppm whereas hydrazide–hydrazone N–H protons (–CONHN=CH–) resonated at 11.59–12.18 ppm [27,28].

The ^{1}H-NMR spectra of **5a**–**c**, which were obtained from reaction of **4a**–**c** with ethyl bromoacetate and anhydrous sodium acetate in absolute ethanol, showed single N_1–H (amide, CONH) and N_2–H (4-thiazolidinone, CONHN=) resonances at 10.19–10.29 and 10.59–10.71 ppm, respectively. The S–CH_2 resonances of **5a**–**c** were observed in the 3.95–4.09 ppm range. In the ^{1}H-NMR spectra of **6a**–**c**, S–CH_2 resonances were

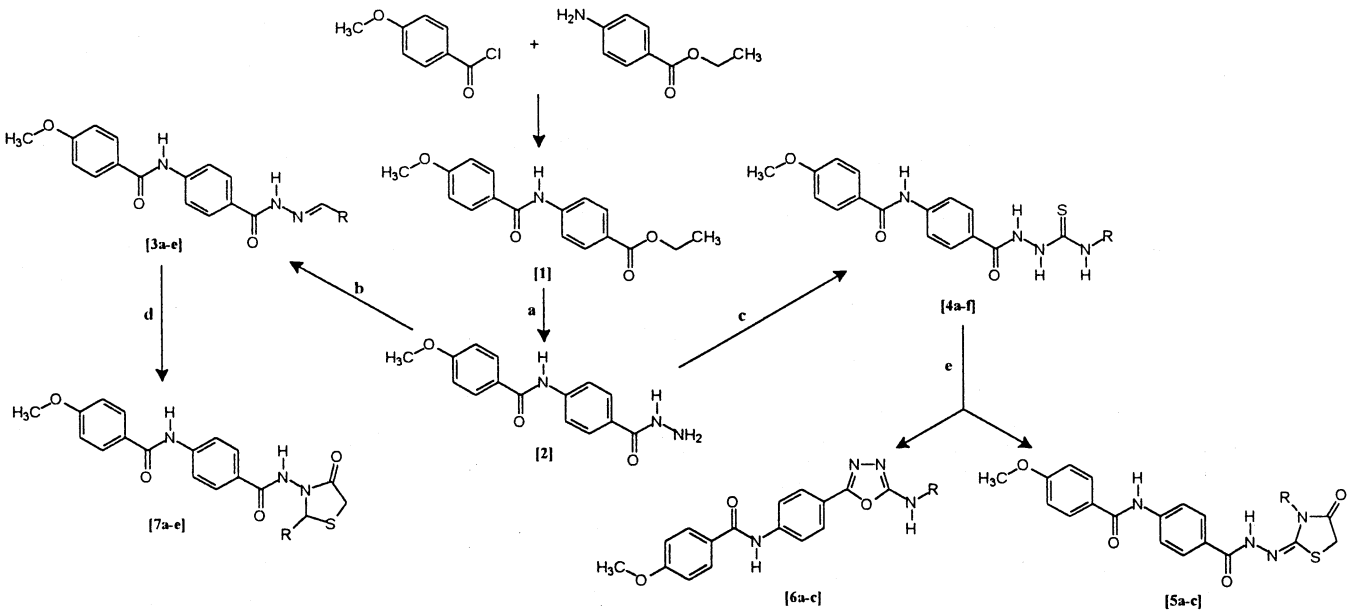

Fig. 2. Key: (a) $H_2N-NH_2 \cdot H_2O$–EtOH; (b) R–CH=O–EtOH; (c) R–NCS–EtOH; (d) HS–CH_2–COOH–benzene; (e) Br–CH_2–$COOC_2H_5$–NaOAc–EtOH.

Table 1
Physical properties and spectral data of **3a–e**; **4e**, **4f**

Compound	R	Molecular formula (molecular weight)	m.p. (°C) (Yield %)	UV, λ_{max} (nm) (log ε)	^{1}H-NMR (DMSO-d_6) (δ, ppm)
3a	5-nitro-2-furyl	$C_{20}H_{16}N_4O_6$ (408.38)	292 (84)	365 (4.30) 300 (4.43) 204 (4.42) 195 (4.16) 192 (4.15)	3.85 (s, 3H, –OCH_3); 7.09 (d, 2H, o-OCH_3, $J = 8.8$ Hz); 7.28 (d, 1H, C3 furan, $J = 3.8$ Hz); 7.81 (d, 1H, C4 furan, $J = 4.0$ Hz); 7.95 (s, 4H, Ar–H); 7.99 (d, 2H, m-OCH_3, $J = 8.8$ Hz); 8.46 (s, 1H, –CH=N–); 10.39 (s, 1H, –CONH–); 12.18 (s, 1H, –CO*H*N=CH–)
3b	–C_6H_4–F(4)	$C_{22}H_{18}FN_3O_3$ (391.40)	294 (95)	315 (4.67) 215 (4.38) 204 (4.45) 191 (4.11)	3.85 (s, 3H, –OCH_3); 7.09–7.99 (m, 12H, Ar–H); 8.46 (s, 1H, –CH=N–); 11.35 (s, 1H, –CONH–); 11.80 (s, 1H, –CO*H*N=CH–)
3c	–C_6H_4–Cl(4)	$C_{22}H_{18}ClN_3O_3$ (407.85)	278 (86)	312 (4.80) 216 (4.56) 205 (4.60) 192 (4.22)	3.85 (s, 3H, –OCH_3); 7.09 (d, 2H, o-OCH_3, $J = 8.8$ Hz); 7.53 (d, 2H, m-Cl, $J = 8.3$ Hz); 7.76 (d, 2H, o-Cl, $J = 8.1$ Hz); 7.93 (s, 4H, Ar–H); 7.99 (d, 2H, m-OCH_3, $J = 8.7$ Hz); 8.46 (s, 1H, –CH=N–); 10.36 (s, 1H, –CONH–); 11.85 (s, 1H, –CO*H*N=CH–)
3d	–C_6H_4–Br(4)	$C_{22}H_{18}BrN_3O_3$ (452.31)	316 (86)	316 (4.22) 221 (3.86) 202 (3.95)	3.85 (s, 3H, –OCH_3); 7.09 (d, 2H, o-OCH_3, $J = 8.8$ Hz); 7.68 (s, 4H, Ar–H); 7.93 (s, 4H, Ar–H); 7.99 (d, 2H, m-OCH_3, $J = 8.7$ Hz); 8.43 (s, 1H, –CH=N–); 10.36 (s, 1H, –CONH–); 11.86 (s, 1H, –CO*H*N=CH–)
3e	–C_6H_3–OH,OC_2H_5(4,3)	$C_{24}H_{23}N_3O_5$ (433.46)	250 (67)	331 (4.91) 306 (4.80) 209 (4.82)	1.37 (t, 3H, –CH_3); 3.85 (s, 3H, –OCH_3); 4.19 (m, 2H, –CH_2CH_3); 6.84–8.01 (m, 11H, Ar–H); 8.33 (s, 1H, –CH=N–); 10.34 (s, 1H, –CONH–); 11.59 (s, 1H, –CO*H*N=CH–)
4e	–C_6H_4–CH_3(4)	$C_{23}H_{22}N_4O_3S$ (434.52)	205 (54)	292 (4.60) 205 (4.66)	2.28 (s, 3H, –CH_3); 3.85 (s, 3H, –OCH_3); 7.08 (d, 2H, o-OCH_3, $J = 8.8$ Hz); 7.13 (d, 2H, o-CH_3, $J = 8.5$ Hz); 7.30–7.94 (m, 6H, Ar–H); 7.98 (d, 2H, m-OCH_3, $J = 8.7$ Hz); 9.62 (s, 1H, –CSNH–); 9.74 (s, 1H, –NHN*H*CS–); 10.33 (s, 1H, –CONH–); 10.42 (s, 1H, –CO*H*NH–)
4f	–C_6H_4–OCH_3(4)	$C_{23}H_{22}N_4O_4S$ (450.52)	204 (83)	292 (4.65) 205 (4.70)	3.74 (s, 3H, –NH–C_6H_4–OCH_3); 3.85 (s, 3H, –CO–C_6H_4–OCH_3); 6.89–7.98 (m, 12H, Ar–H); 9.57 (s, 1H, –CSNH–); 9.68 (s, 1H, –NHN*H*CS–); 10.31 (s, 1H, –CONH–); 10.41 (s, 1H, –CO*H*NH–)

Table 2
Physical properties and spectral data of **5a–c**; **6a–c**; **7a–e**

Compound	R	Molecular formula (molecular weight)	m.p. (°C) (Yield %)	UV, λ_{max} (nm) (log ε)	^{1}H-NMR (DMSO-d_6) (δ, ppm)
5a	$-CH_3$	$C_{19}H_{18}N_4O_4S$ (398.44)	265–269 (58)	297 (4.54) 211 (4.87)	3.17 (s, 3H, –N–CH_3); 3.85 (s, 3H, –O–CH_3); 4.05 (s, 2H, –S–CH_2); 7.07–8.00 (m, 8H, Ar–H); 10.29 (s, 1H, N_1–H); 10.71 (s, 1H, N_2–H)
5c	$-CH_2-CH=CH_2$	$C_{21}H_{20}N_4O_4S$ (424.48)	227–235 (61)	296 (4.58) 223 (4.14)	3.85 (s, 3H, –O–CH_3); 4.09 (s, 2H, –S–CH_2); 5.18 (d, 1H, =C$<_{H}$ cis, $J = 10$ Hz); 5.18 (d, 1H, =C$<^{H}$ trans, $J = 16$ Hz); 5.69–6.08 (m, 1H, –CH=); 7.07–7.99 (m, 8H, Ar–H); 10.29 (s, 1H, N_1–H); 10.59 (s, 1H, N_2–H)
6a	$-C_6H_5$	$C_{22}H_{18}N_4O_3$ (386.41)	295–298 (65)	319 (4.73) 250 (4.31)	3.85 (s, 3H, –O–CH_3); 7.00–8.02 (m, 13H, Ar–H); 10.35 (s, 1H, N_1–H); 10.60 (s, 1H, N_3–H)
6c	$-C_6H_4-OCH_3$(4)	$C_{23}H_{20}N_4O_4$ (416.44)	298–301 (81)	322 (4.34)	3.74 (s, 3H, –C_6H_4–OCH_3); 3.85 (s, 3H, –CO–C_6H_4–O–CH_3); 6.95–8.00 (m, 12H, Ar–H); 10.11–10.57 (d, 2H, N_1–H and N_3–H)
7a	5-nitro-2-furyl	$C_{22}H_{18}N_4O_7S{\cdot}2H_2O$ (518.50)	142–150 (dec.) (55)	295 (4.76) 222 (4.24)	3.85 (s, 3H, –O–CH_3); 3.96, 4.00 (2d and each 1H, –S–CH_2, $J = 16$ Hz); 6.08 (s, 1H, –N–C*H*–S–); 7.03–7.99 (m, 10H, Ar–H); 10.33 (s, 1H, N_1–H); 10.72 (s, 1H, N_2–H)
7c	$-C_6H_4-Cl$(4)	$C_{24}H_{20}ClN_3O_4S$ (481.95)	197–200 (68)	294 (4.67) 212 (4.97)	3.75 (s, 3H, –O–CH_3); 3.84, 3.88 (2d and each 1H, –S–CH_2, $J = 16$ Hz); 5.85 (s, 1H, –N–C*H*–S–); 6.97–7.87 (m, 12H, Ar–H); 10.21 (s, 1H, N_1–H); 10.50 (s, 1H, N_2–H)
7d	$-C_6H_4-Br$(4)	$C_{24}H_{20}BrN_3O_4S$ (526.41)	272–275 (60)	294 (4.62) 227 (4.27)	3.75 (s, 3H, –O–CH_3); 3.84, 3.88 (2d and each 1H, –S–CH_2, $J = 16$ Hz); 5.84 (s, 1H, –N–C*H*–S–); 6.97–7.87 (m, 12H, Ar–H); 10.21 (s, 1H, N_1–H); 10.50 (s, 1H, N_2–H)
7e	$-C_6H_3-OH,OC_2H_5$(4,3)	$C_{26}H_{25}N_3O_6S$ (507.57)	204–213 (dec.) (48)	292 (4.69)	1.33 (t, 3H, C*H*$_3$–CH_2–O–); 3.85 (s, 3H, –O–CH_3); 3.99, 4.02 (2d and each 1H, –S–CH_2, $J = 16$ Hz); 4.07 (q, 2H, CH_3–C*H*$_2$–O); 5.84 (s, 1H, –N–C*H*–S–); 6.97–7.88 (m, 11H, Ar–H); 10.33 (s, 1H, N_1–H); 10.52 (s, 1H, N_2–H)

N_1–H: –CO–NH– (N–H proton of amide); N_2_H: –CO–NH–N<(N–H proton of 4-thiazolidinone); N_3–H: –NH–R (N–H proton bound to 1,3,4-oxadiazole).

not observed. Moreover, elemental analyses revealed that **6a**–**c** had no sulfur atom leading to the conclusion that the structures of these compounds must be different from 4-thiazolidinone.

These observations demonstrated that **6a**–**c** are 1,3,4-oxadiazole. Cesur et al. [29] have been reported previously a similar case. N_1–H (amide, CONH) and N_3–H (1,3,4-oxadiazole, NH) protons of **6a**–**c** were shown at 10.35 and 10.60 (for **6a**), 10.04–10.74, 10.11–10.57 ppm (for **6b** and **6c**), respectively.

The ^{1}H-NMR spectra of **7a**–**e** which were obtained from reaction of **3a**–**e** with mercaptoacetic acid in dry benzene showed single N_1–H (amide, CONH) and N_2–H (4-thiazolidinone, CONHN$<$) resonances at 10.21–10.33 and 10.48–10.72 ppm. In the ^{1}H-NMR spectra, methylene protons of the 4-thiazolidinone ring displayed two signals appearing as doublets at 3.83–3.96 and 3.87–4.00 ppm due to the non-equivalent, geminal methylene protons [10] interacting with the chiral centre at position 2. This phenomenon was not observed with **5a**–**c** lacking the asymmetric carbon. The methin protons of 4-thiazolidinones **7a**–**e** showed resonances at 5.84–6.08 ppm. Methyl protons of the methoxy function gave a singlet at 3.75–3.85 ppm in each case.

EIMS of the selected compounds **5b**, **6b** displayed molecular ions at m/z 412, 400 which confirmed their molecular weights, respectively. Molecular ion was not detected in the mass spectrum of compound **7b**. The fragment ion at m/z 270 peak which formed by the loss of 195 fragment was observed. The major fragmentation pathway appeared by cleavage of CH_3O–C_6H_4–CONH– bonds of amide moiety.

3. Results and discussion

3.1. In vitro evaluation of antimycobacterial activity against Mycobacterium tuberculosis H37Rv

The synthesised compounds **3a**–**e**, **4e**, **4f**, **5a**–**c**, **6a**–**c** and **7a**–**e** were tested in vitro for their antimycobacterial activity. Primary screen was conducted at 6.25 μg mL^{-1} against *M. tuberculosis H37Rv* in BACTEC 12B medium using the BACTEC 460 radiometric system [30]. Rifampin was used as the standard in the tests. Compounds effecting $<90\%$ inhibition in the primary screen (MIC >6.25 μg mL^{-1}) were not evaluated further. Compounds demonstrating at least 90% inhibition in the primary screen were re-tested at lower concentration in a broth microdilution assay alamar Blue. The results of antimycobacterial activity studies are presented in Table 3. The antimycobacterial activity was observed only for compounds **7a** and **7b** with 90 and 98% inhibitions at 6.25 μg mL^{-1}, respectively. Level II assay revealed that the MIC values were not less than 6.25 μg mL^{-1}. These results failed to provide evidence for any reliable structure–activity relationships. However, **7a** and **7b** which had promising antimycobacterial properties could be regarded as lead compounds for further development. Although, all test compounds were dissolved in DMSO, it was observed that the solvent showed no activity in these assays at the level that were used for screening. This was most probably because compounds were initially dissolved in DMSO in the minimal volume, then serially diluted with media to the testing concentration, so there was

Table 3
Antimycobacterial activity of **5a**–**c**, **6a**–**c**, **7a**–**e**, **3a**–**e** and **4e**, **4f**

Compound	R	MIC (μg mL^{-1})	% Inhibition	Level II
5a	–CH_3	>6.25	0	–
5b	–C_2H_5	>6.25	0	–
5c	–CH_2–CH=CH_2	>6.25	0	–
6a	–C_6H_5	>6.25	7	–
6b	–C_6H_4–CH_3(4)	>6.25	0	–
6c	–C_6H_4–OCH_3(4)	>6.25	0	–
7a	5-nitro-2-furyl	<6.25	90	>6.25
7b	–C_6H_4–F(4)	<6.25	98	>6.25
7c	–C_6H_4–Cl(4)	>6.25	40	–
7d	–C_6H_4–Br(4)	>6.25	11	–
7e	–C_6H_3–OH,OC_2H_5(4,3)	>6.25	0	–
3a	5-nitro-2-furyl	>6.25	0	–
3b	–C_6H_4–F(4)	>6.25	2	–
3c	–C_6H_4–Cl(4)	>6.25	3	–
3d	–C_6H_4–Br(4)	>6.25	0	–
3e	–C_6H_3–OH,OC_2H_5(4,3)	>6.25	0	–
4e	–C_6H_4–CH_3(4)	>6.25	0	–
4f	–C_6H_4–OCH_3(4)	>6.25	0	–
Rifampin	–	0.25	98	–

very minimal residual DMSO in the assayed medium. Controls were run in each assay to verify this fact.

3.2. In vitro evaluation of antimicrobial activity

Compounds **3a**–**e**, **4e**, **4f**, **5a**–**c**, **6a**–**c** and **7a**–**e** were screened in vitro for their antimicrobial activity against *Acinobacter baumanii* (18 strains), *A. calcoaceticus* (12 strains), *A. lwoffii* (five strains), *A. johnsonii* (three strains), *Agrobacterium tumefaciens* (10 strains), *A. radiobacter* (eight strains), *A. rubi* (four strains), *Alcaligenes xylosoxydans* (2 starins), *A. faecalis* (two strains), *Bacillus anthracis* (two strains), *B. amyloeiquefaciens* (six strains), *Bacillus anthracis* (two strains), *B. brevis* (13 strains), *B. cereus* (15 strains), *B. licheriformis* (eight strains), *B. magaterium* (three strains), *B. popiliae* (one strain), *B. subtilis* (38 strains), *Brevibacillus agrii* (four strains), *Burkholdria cepecia* (15 strains), *B. gladioli* (three strains), *Clavibacter michiganese* (two strains), *Curtobacterium flaccumfaciens* (one strain), *Enterobacter aerogenes* (eight strains), *E. cloacae* (two strains), *Erwinia carotovora* (three strains), *Escherichia coli* (21 strains), *Edwardsiella spp* (four strains), *Flavobacterium blastinum* (four strains), *Klebsiella pneumoniae* (three strains), *K. oxytoca* (14 strains), *Neisseria spp* (seven strains), *Pantonea agglomerans* (18 strains), *Pseudomonas aeruginosa* (18 strains), *P. syringae pvs.* (52 strains), *Proteus mirabilis* (seven strains), *Serratia liquefaciens* (five strains), *Staphylococcus aureus* (80 strains), *S. epidermis* (43 strains), *Stenotrophomonas maltophilia* (46 strains), *Streptococcus pneumoniae* (10 strains), *S. pyogenes* (41 strains), *Xanthomonas campestris pvs.* (41 strains) for bactericidal and bacteriostatic effects, *Alternaria alternata* (10 strains), *Aspergillus niger* (10 strains), *Fusarium graminearum* (10 strains), *Microsporum canis* (two strains), *Penicillum spp.* (10 strains), *Trichoderma spp.* (10 strains), *Rhizoctonia solani* (10 strains), *Trichophyton spp.* (two strains) at 1 and 0.5 mg mL^{-1} using disc-diffusion method [31]. Penicillin, Cefaclor, Ceftizoxime, Isepamisin, Clindamycin, Erythromycin, Vancomycin, Teicoplanin, Imipenem, Meropenem, Ampicillin-sulbactam, Levofloksasin for gram (+) bacteria and Isepamisin, Amikacin, Netilmicin, Ceftriaxone, Cefoperazone, Cefepime, Ceftizoxime, Trimethoprim-Sulphametoxazol, Imipenem, Meropenem, Aztreonam, Ampicillin, Ofloxacin for gram (−) bacteria were used as standards in the tests. All compounds were found inactive against tested bacteria and fungi, whereas **3a** and **7a** showed varying degrees of inhibition against several bacteria and fungi. Compound **3a** is more active against gram (+) than gram (−) bacteria and has a better antibacterial activity than compound **7a**. The results of antimicrobial activity are given in Tables 4 and 5.

4. Experimental

4.1. Chemistry

Benzocaine, Et_2O, BzCl, ethyl and allyl isothiocyanates were purchased from Merck Company. All the other chemicals used in the experiments were purchased from Fluka. All m.p. were recorded on a Büchi 530 m.p. apparatus and uncorrected. UV spectra were recorded on a Shimadzu UV 2100S spectrophotometer (1 mg/100 mL in EtOH). ^{1}H-NMR spectra were obtained on a Bruker AVANC-DPX 400 instrument. Elemental analyses were performed on a Carlo Erba 1106 instrument and the results were in acceptable range.

4.1.1. Preparation of ethyl 4-(4-methoxybenzoylamino)benzoate (*1*) and 4-(4-methoxybenzoylamino)benzoyl hydrazine (*2*)

These compounds were prepared according to the method described by Rollas et al. [24].

4.1.2. Synthesis of N^1-[4-(4-methoxybenzamido)-benzoyl]-N^2-substituted alkylidene hydrazines (*3a*–*e*) and 1-[4-(4-methoxybenzamido)-benzoyl]-4-substituted phenylthiosemicarbazides (*4a*–*f*)

A solution of 0.01 mol of 4-(4-methoxybenzoylamino)benzoyl hydrazine (**2**) and equimolar amount of appropriate aldehyde (for **3a**–**e**)/appropriate isothiocyanate (for **4a**–**f**) in 100 mL of EtOH was heated under reflux for 2 h. The precipitate obtained was filtered-off, washed with water and cleaned twice with boiling EtOH. Physical and spectral data of **3a**–**e** and **4e**, **4f** are given in Table 1. Compounds **4a**–**d** were previously described [24] [**4a** (R = –CH_3) m.p. 234–235 °C, lit. [24] 227–230 °C; **4b** (R = –C_2H_5) m.p. 230, lit. [24] 231 °C; **4c** (R = –CH_2–CH=CH_2) m.p. 218, lit. [24] 216–218 °C and **4d** (R = –C_6H_5) m.p. 249–250 °C, lit. [24] 250 °C].

4.1.3. Synthesis of 2-[4-(4-methoxybenzoylamino)-benzoylhydrazono]-3-alkyl-4-thiazolidinones (*5a*–*c*)

0.01 mol of appropriate thiosemicarbazide **4a**–**c** and 0.011 mol of ethyl bromoacetate were refluxed in 30 mL of absolute EtOH in the presence of 0.04 mol of anhydrous NaOAc for 9 h. The reaction mixture was cooled, diluted with water and allowed to stand overnight. The solid precipitated was washed with water, dried and washed with hot EtOH to give **5a**–**c**. Analysis for **5b**: R: –C_2H_5. $C_{20}H_{20}N_4O_4S$. Molecular weight: 412.40. m.p. 247–250 °C. Yield: 67%. UV (EtOH) λ_{max} (nm) (log ε): 296 (4.70), 223 (4.24). ^{1}H-NMR (400 MHz, DMSO-d_6), ppm: 1.11 (t, 3H, CH_3–CH_2–); 3.67 (q, 2H, CH_3–CH_2–); 3.76 (s, 3H, –O–CH_3); 3.95 (s, 2H, –S–CH_2); 6.99–7.89 (m, 8H, Ar–H); 10.19 (s, 1H, N_1–H); 10.63 (s, 1H, N_2–H). ^{13}C-NMR (100.6 MHz, DMSO-d_6), ppm: 13.02

Table 4
Antimicrobial activity of **3a**–**e** and **4e**, **4f**

	Number of strains tested	Bactericidal effect (1 mg mL^{-1})	Bactericidal effect (0.5 mg mL^{-1})	Bacteriostatic effect (1 mg mL^{-1})	Bacteriostatic effect (0.5 mg mL^{-1})
		3a	**3a**	**3a**	**3a**
Acinetobacter baumanii	18	–	–	–	–
Acinetobacter calcoaceticus	12	–	–	–	–
Acinetobacter lwoffii	5	–	–	–	–
Acinetobacter johnsonii	3	–	–	–	–
Agrobacterium tumefaciens	10	–	–	–	–
Agrobacterium radiobacter	8	–	–	–	–
Agrobacterium rubi	4	–	–	–	–
Bacillus anthracis	2	7–9 mm	3–5 mm	–	–
Bacillus amyloeiquefaciens	6	7–11 mm	5–7 mm	–	–
Bacillus brevis	13	6–8 mm	3–5 mm	–	–
Bacillus cereus	15	7–9 mm	4–5 mm	–	–
Bacillus licheriformis	8	7–11 mm	–	–	–
Bacillus magaterium	3	4–7 mm	3–4 mm	–	–
Bacillus popilliae	1	7 mm	4 mm	–	–
Bacillus substilis	38	6–13 mm	4–8 mm	–	–
Brevibacillus agrii	4	7–10 mm	4–7 mm	–	–
Burkholdria cepacia	15	–	–	–	–
Burkholdria gladioli	3	–	–	–	–
Clavibacter michiganense	2	3–4 mm	1–2 mm	–	–
Curtobacterium flaccumfaciens	1	2 mm	–	–	–
Enterobacter aerogenes	8	–	–	2–3 mm/3 strain; –/5 strain	–
Enterobacter cloacae	2	–	–	–	–
Erwinia carotovora	3	–	–	–	–
Escherichia coli	21	–	–	1–2 mm/7 strains; –/14 strains	–
Flavobacterium blastinum	4	–	–	–	–
Klebsiella pneumoniae	3	–	–	–	–
Neisseria spp	7	5–9 mm	3–4 mm	–	–
Pantoea agglomerans	18	–	–	–	–
Pseudomonas aeruginosa	14	–	–	–	–
Pseudomonas syringae pvs.	52	–	–	–	–
Staphylococcus aureus	80	5–9 mm/74 strain; –/6 strains	2–4 mm/74 strain; –/6 strains	–	–
Staphylococcus epidermis	43	4–10 mm	2–3 mm	–	–
Stenotrophomonas maltophilia	46	–	–	–	–
Streptococcus pneumoniae	10	3–7 mm/8 strains; –/2 strains	1–2 mm/7 strains; –/3 strains	–	–
Streptococcus pyogenes	41	6–9 mm/35 strains; –/6 strains	2–4 mm/31 strains; –/10 strains	–	–
Xanthomonas campestris pvs.	140	–	–	2–4 mm/ 22 strains; –/118 strains	–
Total	655	–	–		

*, For **3b**, **3c**, **3d** and **3e** effect bacteriostatic against *Escherichia coli*: 2–3 mm (1 mg mL^{-1} concentration); *, for **4e** and **4f** effect bacteriostatic against *Enterobacter aerogenes*, *E. coli Streptococcus pyogenes and Xanthomonas campestris*: 2–4 mm (1 mg mL^{-1} concentration).

(N–CH_2–CH_3); 33.1 (S–CH_2); 38.4 (N–CH_2–CH_3); 56.32 (OCH_3);114.53; 120.32; 127.52; 129.06; 130.61; 143.17 (Aromatic C); 156.87 (4-thiazolidinone C=N); 163.12 (hydrazone C=O); 165.16 (amide C=O); 172.5 (4-thiazolidinone C=O). EIMS (70 eV, m/z) (%): 412 [M^+] (14.74); 338 (3.78); 271 (2.95); 270 (7.95); 254 (14.10); 135 (100); 120 (2.12); 107 (6.38); 92 (7.40).

*4.1.4. Synthesis of 2-[4-(4-methoxybenzoylamino)-phenyl]-5-(substituted phenyl) amino-1,3,4-oxadiazoles (**6a**–**c**)*

Compounds **6a**–**c** were obtained from **4d**–**f** as described for **4a**–**c**. Analysis for **6b**: R: –C_6H_4–CH_3 (**4**). $C_{23}H_{20}N_4O_3$. Molecular weight: 400.44. m.p. 299–

Table 5
Antimicrobial activity of **5a–c**, **6a–c** and **7a–e**

	Number of strains tested	Bactericidal effect (1 mg mL^{-1})	Bactericidal effect (0.5 mg mL^{-1})	Bacteriostatic effect (1 mg mL^{-1})	Bacteriostatic effect (0.5 mg mL^{-1})
		7a	**7a**	**7a**	**7a**
Acinetobacter baumanii	18	–	–	–	–
Acinetobacter calcoaceticus	12	–	–	–	–
Acinetobacter lwoffii	5	–	–	–	–
Acinetobacter johnsonii	3	–	–	–	–
Agrobacterium tumefaciens	10	–	–	–	–
Agrobacterium radiobacter	8	–	–	–	–
Agrobacterium rubi	4	–	–	–	–
Bacillus anthracis	2	3–5 mm	2–3 mm	–	–
Bacillus amyloliquefaciens	6	5–7 mm	3–4 mm	–	–
Bacillus brevis	13	4–7 mm	2–3 mm	–	–
Bacillus cereus	15	6–9 mm	4–5 mm	–	–
Bacillus licheriformis	8	5–9 mm	–	–	–
Bacillus magaterium	3	5–7 mm	3–4 mm	–	–
Bacillus popilliae	1	5 mm	3 mm	–	–
Bacillus substilis	38	4–8 mm	3–5 mm	–	–
Brevibacillus agrii	4	3–7 mm	2–3mm	–	–
Burkholdria cepacia	15	–	–	–	–
Burkholdria gladioli	3	–	–	–	–
Clavibacter michiganense	2	–	–	–	–
Curtobacterium flaccumfaciens	1	–	–	–	–
Enterobacter aerogenes	8	–	–	–	–
Enterobacter cloacae	2	–	–	–	–
Erwinia carotovora	3	–	–	–	–
Escherichia coli	21	2–3 mm/ 15 strains; −/6 strains	–		–
Flavobacterium blastinum	4	–	–	–	–
Klebsiella pneumoniae	3	–	–	–	–
Neisseria spp	7	–	–	–	–
Pantoea agglomerans	18	–	–	–	–
Pseudomonas aeruginosa	14	–	–	–	–
Pseudomonas syringae pvs.	52	–	–	–	–
Staphylococcus aureus	80	4–7 mm	2–3 mm	–	–
Staphylococcus epidermis	43	6–9 mm	2–4 mm	–	–
Stenotrophomonas maltophilia	46	–	–	2–3 mm/ 7 strains; −/39 strains	–
Streptococcus pneumoniae	10	3–5 mm/ 5 strains; −/5 strains	1–2 mm/ 5 strains; −/5 strains	–	–
Streptococcus pyogenes	41	5–7 mm	2–3 mm	–	–
Xanthomonas campestris pvs.	140	–	–	3–4 mm/ 39 strains; −/101 strains	–
Total	655	–	–	–	

*, Bacteriostatic effect of **5a**, **5b**, **5c**, **6a**, **6b** and **6c** against *E. coli* and *Enterobacter aerogenes*: 2–5 mm (1 mg mL^{-1} concentration); *, bacteriostatic effect of **7b**, **7c**, **7d** and **7e** against *E. coli* and *Xanthomonas campestris*: 2–3 mm (1 mg mL^{-1} concentration); standards used for Gram(+) bacteria: Penicillin, Cefaclor, Ceftizoxime, Isepamisin, Clindamycin, Erythromycin, Vancomycin, Teicoplanin, Imipenem, Meropenem, Ampicillin-sulbactam, Levofloksasin (5–15 mm zone); standards used for Gram(−) bacteria: Isepamisin, Amikacin, Netilmicin, Ceftriaxon, Cefoperazon, Cefepim, Ceftizoxime, Trimethoprim-Sulphametoxazol, Imipenem, Meropenem, aztreonam, Ampicillin, Ofloxacin (5–15 mm zone).

303 °C. Yield: 76%. UV (EtOH) λ_{max} (nm) (log ε): 321 (4.57), 254 (4.23). ^{1}H-NMR (400 MHz, DMSO-d_6), ppm: 2.27 (s, 3H, –C_6H_4–CH_3); 3.85 (s, 3H, –O–CH_3); 7.07–8.01 (m, 12H, Ar–H); 10.04–10.74 (d, 2H, N_1–H and N_3–H). ^{13}C-NMR (100.6 MHz, DMSO-d_6), ppm: 21.18 (C_6H_4–CH_3); 56.31 (OCH_3);114.53; 117.92; 119.43; 121.24; 127.03; 127.47; 130.33; 130.62; 131.52; 137.08 (Aromatic C); 142.62; 158.43 (oxadiazole C=N); 166.07 (amide C=O). EIMS (70 eV, m/z) (%): 400 [M^+] (10.00); 135(100); 107 (9.50).

*4.1.5. Synthesis of 2-substituted-3-{[4-(4-methoxybenzoylamino)benzoyl]amino}-4-thiazolidinones (**7a–e**)*

A mixture of **3a–e** (0.01 mol) and mercaptoacetic acid (0.01 mol) was refluxed in dry C_6H_6 (50 mL) using a *Dean–Stark* water separator. Excess C_6H_6 was evaporated in vacuo. The resulting residue was triturated with saturated $NaHCO_3$ solution until CO_2 evolution ceased. The solid was washed with water, dried and recrystallised from ethanol–water. Anal. for **7b**: R: $-C_6H_4F$(4). $C_{24}H_{20}FN_3O_4S$. Molecular weight: 465.50. m.p. 257–258 °C. Yield: 74%. UV (EtOH) λ_{max} (nm) (log ε): 293 (4.53), 222 (4.06). ^{1}H-NMR (400 MHz, DMSO-d_6), ppm: 3.76 (s, 3H, –O–CH_3); 3.83, 3.87 (2d and each 1H, –S–CH_2, $J = 15.9$ Hz); 5.86 (s, 1H, –N–C*H*–S–); 6.98–7.87 (m, 12H, Ar–H); 10.21 (s, 1H, N_1–H); 10.48 (s, 1H, N_2–H). ^{13}C-NMR (100.6 MHz, DMSO-d_6), ppm: 30.19 (S–*C*H_2); 56.32 (O*C*H_3); 61.94 (S–*C*H–N); 114.52; 116.20; 116.41; 120.24; 126.70; 127.36; 129.26; 130.03; 130.89; 130.98; 135.45; 143.90 (Aromatic C); 162.99 (hydrazone C=O); 166.06 (amide C=O); 170.04 (4-thiazolidinone C=O). EIMS (70 eV, m/z) (%): 270 [$M^+ - 195$] (2.53); 254 (2.33); 197 (3.92); 150 (3.20); 135 (100); 123 (2.93); 122 (12.26); 120 (3.73); 107 (5.86); 92 (7.07).

4.2. Microbiology

4.2.1. In vitro evaluation of antimycobacterial activity against M. tuberculosis H37Rv

Primary screen was conducted at 6.25 μg mL^{-1} against *M. tuberculosis H37Rv* in BACTEC 12B medium using the BACTEC 460 radiometric system [30]. Compounds effecting $< 90\%$ inhibition in the primary screen (MIC > 6.25 μg mL^{-1}) were not evaluated further. Compounds demonstrating at least 90% inhibition in the primary screen were re-tested at lower concentration (MIC) in a broth microdilution assay alamar Blue. The MIC was defined as the lowest concentration inhibiting 99% of the inoculum.

4.2.1.1. BACTEC radiometric method of susceptibility testing. Inocula for susceptibility testing were either from a positive BACTEC isolation vial with a growth index (GI) of 500 or more, or a suspension of organisms isolated earlier on a conventional medium.

The culture was well mixed with a syringe and 0.1 mL of a positive BACTEC culture was added to each of the vials containing the test drugs. The drug vials contained rifampin (0.25 μg mL^{-1}). A control vial was inoculated with a 1:100 dilution of the culture. A suspension equivalent to a Mc Farland No. 1 standard was prepared in the same manner as a BACTEC positive vial, when growth from a solid medium was used.

Each vial was tested immediately on a BACTEC instrument to provide CO_2 in the headspace. The vials were incubated at 37 °C and tested daily with a BACTEC instrument. When the GI in the control reads at least 30, the increase in GI (ΔGI) from the previous day in the control was compared with that in the drug vial. The following formula was used to interpret results:

$$\Delta GI \text{ control} > \Delta GI \text{ drug} = \text{susceptible}$$

$$\Delta GI \text{ control} < \Delta GI \text{ drug} = \text{resistant}$$

If a clear susceptibility pattern (the difference of ΔGI of control and the drug bottle) was not seen at the time the control GI is 30 the vials were read for 1 or 2 additional days to establish a definite pattern of ΔGI differences.

4.2.2. Antimicrobial activity

In the present study, disc-diffusion method was utilised for the determination of antibacterial and antifungal activities of the synthesised compounds. Whatman filter paper discs were impregnated with test compounds, dissolved in DMSO at 0.5 and 1.0 mg mL^{-1}. Tryptic Soy Agar (TSA) and Potato Dextrose Agar (PDA) were used as growth media for bacterial and fungal strains, respectively. The bacteria to be tested were initially grown as 24–48 h fresh cultures in TSA. Each bacterial strain was then suspended in 0.1 M buffer; diluted to an inoculum size to have an optical density of 0.1 at 600 nm (ca. 10^8 CFU mL^{-1}) and 0.1 mL of these suspensions were disseminated onto plates containing TSA. After drying the suspensions keeping the plates in a sterilizer, paper discs impregnated with test compounds were placed onto plates maintaining equal distance between each disc (eight discs/plate). Following incubation of the plates at 26 and 30 °C for 24–48 h, inhibition zones surrounding the test discs were measured. Test plates were then followed up for further 7 days in order to determine whether any compound was bactericidal or bacteriostatic.

For the determination of antifungal activity, fungal isolates were cultivated in PDA for 4–10 days. A micelle disc, obtained from pure fungal culture, at the centre of test plates and discs containing the test compounds at the margins in equal distance from micelle disc were placed. These plates were then incubated at 25 °C for 4–10 days. Antifungal activity of the test compounds was evaluated measuring inhibition zones when fungal micelles covered up the plate surface.

Paper discs with only DMSO were used as negative controls. In all determinations, tests were performed duplicate and the results were reported as mean of these values.

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