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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis and molecular modelling of novel 4-thiazolidinones of potential activity against Gram positive bacteria

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Abstract A series of novel 4-thiazolidinones (**2–21**) incorporating 2-(2,4,5-trichlorophenoxy)propanamide was synthesised. Reaction of 2-(2,4,5-trichlorophenoxy)propanohydrazide (**1**) with the corresponding carbonyl compounds afforded 2-(2,4,5-trichlorophenoxy)propanehydrazide hydrazones (**2–11**) which upon reaction with thioglycolic acid revealed 4-thiazolidinone derivatives (**12–21**). Structure elucidation of the synthesised compounds was done based on analytical and spectral data. The newly synthesised compounds were evaluated for their antimicrobial activity. Compounds **13** and **17** showed the equipotent activity with MIC value 6.25 μg ml^{-1} compared with chloramphenicol as reference drug. Docking studies of the promising compounds was done on *MurB* using Dock6.4 docking program to study their observed activity.

Keywords 4-Thiazolidinones · Hydrazide–hydrazones · Synthesis · Dock · Antibacterial

Introduction

One of the challenging dilemma in the medical community is the treatment of infectious diseases in hospital-based healthcare because of a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens with particular relevance for Gram positive bacteria (Dessen *et al.*, 2001; Muroi *et al.*, 2004; Pfeltz and Wilkinson, 2004; Roberts, 2004; Tenover and McDonald, 2005). These resistant microorganisms cause infectious diseases which are considered as an important challenge to the medical community and need for an effective therapy has led to an increasing search for novel antimicrobial agents (Chikhalia and Vashi, 2009). Microorganism resistance to antimicrobials threatens the health of many throughout the world, since both old and new infectious diseases remain a formidable public health threat. One of the most interesting areas of research is the search for compounds that combat resistant bacteria. Thiazolidinones were found to possess antibacterial activity (Fuloria *et al.*, 2009; Kumar *et al.*, 2012; Mistry and Jauhari, 2012; Solankee *et al.*, 2012). Naturally occurring molecules containing thiazolidinone moiety showed important antibiotic, immunosuppressive and antitumor activities (Vagdevi *et al.*, 2006; Badorc *et al.*, 1997; Rzasa *et al.*, 1998; Zhong, 2011). Thiazolidinones also, possess a wide spectrum of activities like antifungal (Karali *et al.*, 1998; Fahmy, 2001, Khan and Yusuf, 2009), anticonvulsant (Ergenc and Capan, 1994; Capan *et al.*, 1996), COX-1 inhibitors (Look *et al.*, 1996), antituberculosis (Bukowski *et al.*, 1998; Ulusoy, 2002; Babaoglu *et al.*, 2003) and antihistaminic (Diurno *et al.*, 1992) activity. Thiazolidinones containing β-lactam ring inhibit the biosynthesis of the peptidoglycan polymer essential for cell wall of bacteria. *MurB* enzyme is a unique target for antibacterial activity of thiazolidinone (Andres *et al.*, 2000a, b). Thiazolidinones **I** were reported to be potent inhibitor of *MurB* (Andres *et al.*, 2000a, b; Bronson *et al.*, 2003). This activity, coupled with the design

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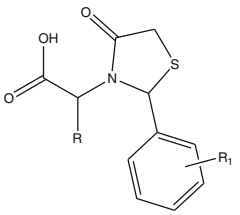
principles of the thiazolidinones, supports the postulate that 4-thiazolidinones may be recognized as diphosphate mimics by a biological selector (Andres *et al.*, 2000a, b). On the basis of crystal structure analysis of the *MurB* enoylpyruvate–UDP-*N*-acetylglucosamine (EP–UNAG) complex, it was found that the carboxylate of the substrate interacts with residues Arg159 and Glu325 and could be responsible for transition state stabilization whereas the diphosphate moiety of the substrate interacts with residues Tyr190, Lys217, Asn233 and Glu288. Thus, templates were chosen that contained functional surrogates of the diphosphate with side-chains oriented to occupy space similar to the glucosamine and uridine moieties as potential *MurB* inhibitors. The 4-thiazolidinones met these criteria and a series of compounds were synthesised (Andres *et al.*, 2000a, b).



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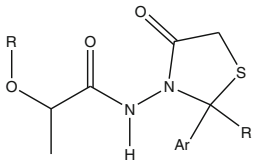


II

In view of these findings and in continuation of our work on the synthesis of antimicrobial agents of pharmaceutical interest (Radwan and Hussein, 2006; Hyallah and Radwan, 2007; Mostafa *et al.*, 2008), the rational approach to lead discovery has prompted a better insight in developing new thiazolidinone derivatives, with general formula **II**, based on the structural formula **I** to explore substitution effect, at 2- and 3-position of the thiazolidinone ring, on the antimicrobial activity.

Results and discussion

Synthesis

The key intermediate compound **1** was prepared from 2-(2,4,5-trichlorophenoxy)propanoic acid by the ester formation followed by hydrazinolysis. The target compounds, **2–11**, were synthesised by the reaction of compound **1** with the corresponding carbonyl compound.

2-(2,4,5-Trichlorophenoxy)-*N*-(4-oxo-2-substitutedthiazolidin-3-yl)propanamide derivatives (**12–21**) were obtained via reaction of the corresponding compounds **2–11** with thioglycolic acid, Scheme 1. Figure 1 shows that the hydrazone undergoes attack by sulphur nucleophile, followed by intramolecular cyclization on elimination of water (Bolognese *et al.*, 2004). Structures of the synthesised compounds were verified on the bases of spectral and elemental methods of analyses and were consistent with the proposed structures. All spectral data are in accordance with the assumed structures.

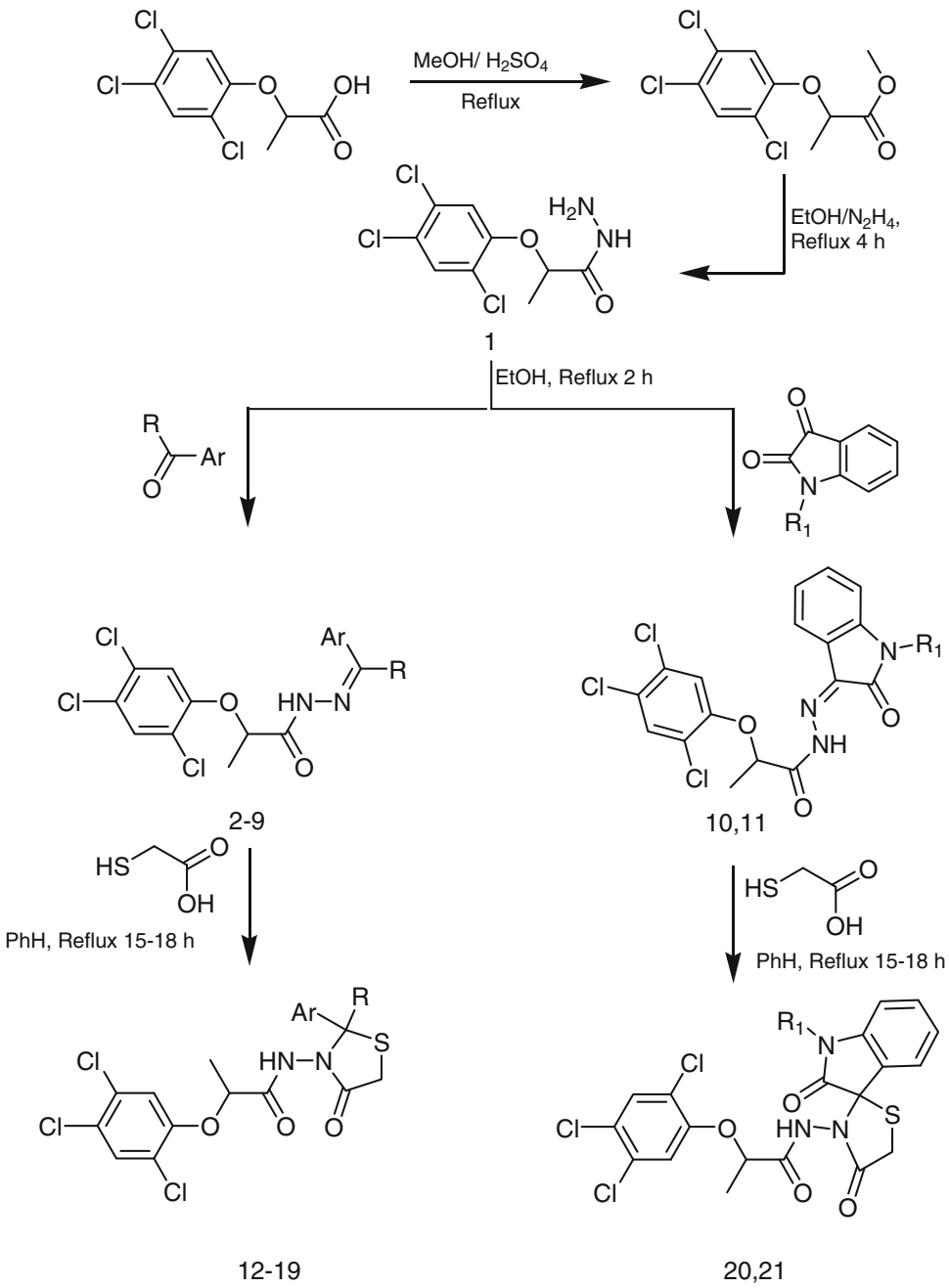
The most differentiating stretching bands in IR spectra are shown at the range of 1,624–1,600 cm^{-1} for the hydrazone C=N group of compounds, **2–11**, and at the range of 1,722–1,692 cm^{-1} for the carbonyl functionality of the 4-thiazolidinone moiety of compounds **12–21**. Generally, all the synthesised compounds **2–21** showed carboxamide group at the range of 1,687–1,664 and 3,256–3,164 cm^{-1} for the carbonyl and amide functionalities, respectively. Furthermore, aliphatic C–H stretching around 2,994–2,904 cm^{-1} and aromatic C–H stretching around 3,100–3,031 cm^{-1} are also observed. ^{1}H and ^{13}C NMR signals of compounds (**2–21**) showed a similar trend in the chemical shift of the common part of the molecular backbone. ^{1}H-NMR spectra of compounds **2–7** indicated the presence of a singlet at δ 8.1–8.43 ppm which could be assigned to CH=N. Compound **8** showed a characteristic signal at δ 2.25 and 2.31 ppm corresponding to methyl group of p-tolyl moiety and methyl group of ethylidene moiety, respectively. Compound **9** showed a characteristic signal at δ 2.31 ppm due to methyl group of ethylidene moiety. Compounds **10** and **11** showed characteristic signals at δ 6.99–7.96 ppm due to isatin moiety. ^{1}H-NMR spectra of thiazolidinones **12–21** revealed the disappearance of imino group and appearance of a signal at δ 3.24–3.71 which could be assigned for the methylene C-5 protons of the thiazolidinone ring. In addition, compounds **12–17** showed a signal at δ 5.77–6.07 due methine C-2 proton of the thiazolidinone ring.

^{13}C-NMR spectra of compounds hydrazone compounds **2–11** showed a characteristic signal at δ 156.1–167.1 ppm due to hydrazone C=N, while the thiazolidinone compounds **12–21** showed characteristic signals at δ 34.5–41.66, 53.01–72.34 and 165.88–170.46 ppm due to the thiazolidinones C-5, C-2 and C-4, respectively.

Mass spectra and elemental analysis were found in agreement with the suggested chemical structures of the synthesised compounds.

Antimicrobial evaluations

Compounds (**2–21**) were evaluated for their in vitro antimicrobial activity against Gram positive bacteria: *S. aureus* (ATCC 25923), *B. subtilis* (ATCC 10400) and Gram negative bacteria: *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27855), *Shig. Sonnei* (ATCC 11060) and fungal strains: *C. albicans* (ATCC 10231). Antimicrobial activity was assessed by serial twofold dilution technique (Madhukar *et al.*, 2009). Chloramphenicol was used as a standard drug for antibacterial activity and miconazole was used as a standard drug for antifungal activity. Table 1 lists the MIC values of the tested compounds.



Scheme 1 Adopted synthetic pathways of compounds **2**–**21**. **2**, **12** R=H, Ar=$C_6H_4NO_2$-3; **3**, **13** R=H, Ar=C_4H_3S-2; **4**, **14** R=H, Ar=C_4H_2S–CH_3–5; **5**, **15** R=H, Ar=C_4H_2O–CH_3–5; **6**, **16** R=H, Ar=C_5H_4N-3; **7**, **17** R=H, Ar=C_5H_4N-4; **8**, **18** R=CH_3, Ar=C_6H_4–CH_3-4; **9**, **19** R=CH_3, Ar=C_6H_4-Cl-4; **10**, **20** R_1=H; **11**, **21** R_1=CH≡C–CH_2

The thiazolidinone derivatives (1**2**–**21**) were found inactive against fungi strains and Gram negative bacteria, while they exhibited promising antibacterial activity against Gram positive bacteria strains. Compounds **13** and **17** were found equipotent to chloramphenicol as growth inhibitors of the tested Gram positive bacteria. Compounds **12**, **14**–**16**, **18** and **19** were found either equipotent or slightly less potent than chloramphenicol as growth inhibitors of the tested Gram positive bacteria, while compounds **20** and **21** showed a weak activity. On the other hand, compounds (**2-11**) did not show any activity against all the species tested.

Antimycobacterium tuberculosis activity

Primary screening of compounds (**2**–**21**) for their in vitro antituberculosis against *M. tuberculosis* H37Rv using microdilution assay, the Microplate Alamar Blue Assay (MABA) (Collins and Franzblau, 1997). None of the synthesized compounds exhibited antimycobacterial activity.

Docking procedure

In order to investigate the possible interactions between our newly synthesised compounds and the active site of the

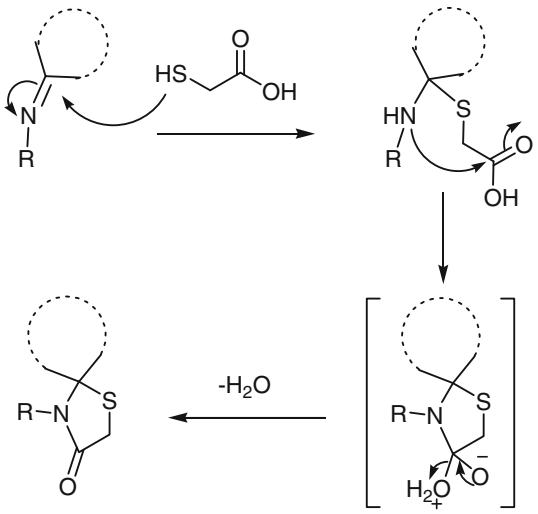


Fig. 1 Mechanism of formation of 1,3-thiazolidin-4-ones from imine

MurB enzyme, a docking process were undertaken using Dock6.4 (Lang *et al.*, 2009). In the docking procedure, the coordinates of UDP-*N*-acetylmuramate dehydrogenase (*E. coli* MurB) enzyme X-ray structure, complexed with its co-crystallised ligand naphthyl tetronic acid inhibitor, (2Q85) was taken from the Protein Data Bank (Mansour *et al.*, 2007). The RMSD value difference of 0.53 Å of the pose from the non-restricted redocking of the naphthyl tetronic acid inhibitor, MurB-bound X-ray ligand, structure itself also confirmed the approach (Fig. 2). The binding site includes hydrophobic pocket delineated by the side chains of Lys 217, Leu 218, Ala 265, Glu 288, Leu 290, and the binding site which is enriched with hydrogen bond donor groups represented by Arg 159, Arg 214, Asn 233, Glu 325 and Tyr 190 that surrounds the functional groups of the oxalane ring of naphthyl tetronic acid inhibitor, X-ray ligand (Fig. 2).

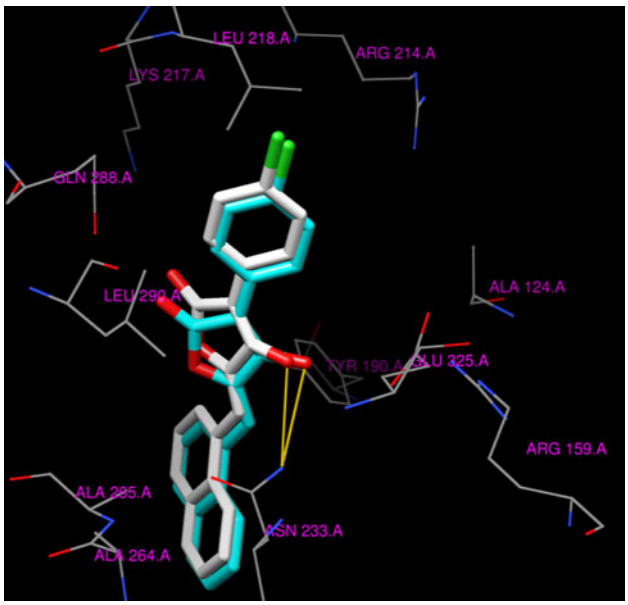


Fig. 2 Naphthyl tetronic acid from 2Q85 (X-ray *white*, docked *cyan*) oriented in UDP-*N*-acetylmuramate dehydrogenase (*MurB*) binding site (hydrogen bonds are displayed in *orange* cylinder) (Color figure online)

The docking poses belonging to our newly synthesised anti-Gram positive bacteria active compounds (Figs. 3, 4) suggested that hydrogen bonding between the carbonyl oxygen of the thiazolidinone ring and the amino group of Asn 233 stabilized the aromatic ring (4-pyridinyl) group over the thiazolidinone ring system in parallel orientation between its pi system and the Ala 264, Ala 265 and Leu 290 with hydrophobic interaction between hydrophobic side chains of these amino acids and the electronic system of the pi system. However, the trichlorophenyl moiety is generally oriented in the hydrophobic pocket surrounded by the side chains of Ala 124, Arg 214, Lys 217 and Leu 218. The propionamide moiety is stabilized by the

Table 1 Minimum inhibitory concentration in μg ml^{-1} of compounds **12–21** against selected microbial strains

Compd. no.	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Salmonella typhimurium*	*Shigella sonnei*	*Pseudomonous aeruginosa*	*Candida albicans*
12	12.5	12.5	–	–	–	–	–
13	6.25	6.25	–	–	–	–	–
14	12.5	12.5	–	–	–	–	–
15	12.5	12.5	–	–	–	–	–
16	6.25	12.5	–	–	–	–	–
17	6.25	6.25	–	–	–	–	–
18	12.5	6.25	–	–	–	–	–
19	12.5	12.5	–	–	–	–	–
20	25	50	–	–	–	–	–
21	50	50	–	–	–	–	–
Chloramph	6.25	6.25	12.5	25	25	–	–
Miconazole	–	–	–	–	–	3.12	3.12

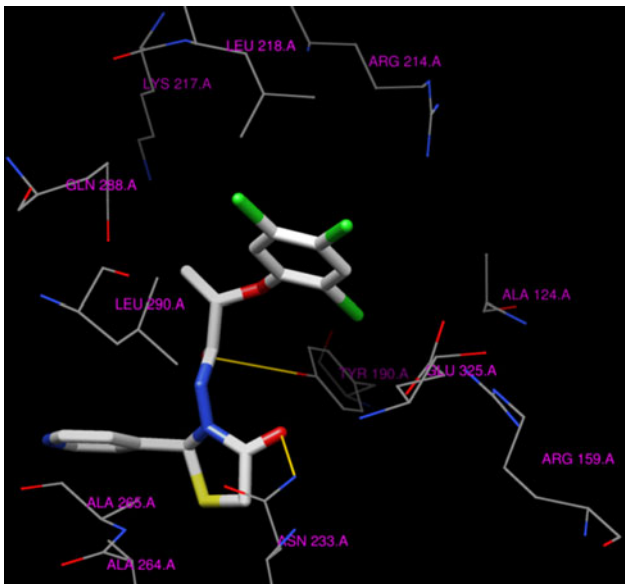


Fig. 3 Compound **17** (coloured *white*) docked in *MurB* binding site (hydrogen bonds are displayed in *orange* cylinder) (Color figure online)

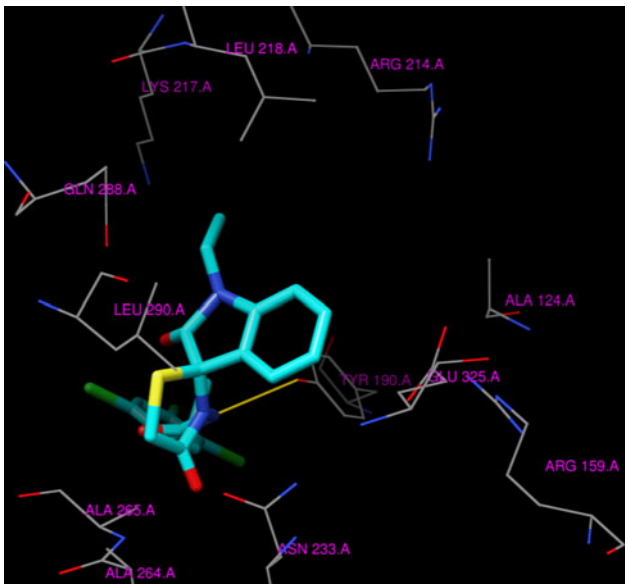


Fig. 5 Compound **21** (coloured *cyan*) docked in *MurB* binding site (hydrogen bond is displayed in *orange* cylinder) (Color figure online)

hydrophobic interaction between the side chain of Leu 290 and the methyl group of propionamide moiety and, in some cases, hydrogen boding between the amide oxygen atom and the hydroxyl group of Tyr 190. The docking poses belonging to our newly synthesised compounds with thiazolidinone ring in spiro structure with heterocyclic ring (anti-Gram positive bacteria weak active compounds **20** and **21**, Fig. 5) failed to be oriented inside the proposed binding pocket.

Although the generated docking poses illustrated a parallelism between MIC values against the tested Gram positive bacteria strains and compound interactions with the surrounding environment regarding side chain changes in general, more new analogues are suggested to be synthesised with its in vitro evaluation against *MurB* enzyme for further molecular modelling studies would be the best option for verification.

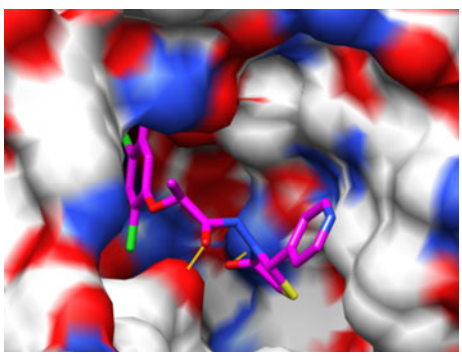


Fig. 4 Binding site surface with docked, compound 17 (coloured *magenta*, hydrogen bond coloured *orange*) (Color figure online)

Experimental

Synthesis

Melting points were determined on Barnstead 9001 Electrothermal melting point apparatus using open capillary tubes and are uncorrected. IR spectra were obtained, as KBr discs, on a Perkin Elmer FT-IR spectrophotometer at the Research Center, College of Pharmacy, King Saud University, Saudi Arabia. The data are given in ν_{max} (cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded in either DMSO-d_6 or $CDCl_3$ on a Bruker NMR spectrophotometer operating at 500 MHz for ^{1}H and 125.76 MHz for ^{13}C; the chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane (TMS) used as internal standard. Mass spectra were taken on a Varian 320-MS spectrometer at the Research Center, College of Pharmacy, King Saud University, Saudi Arabia. Mass spectral data were given as *m*/*z* (intensity %). Elemental analysis was performed on a Perkin Elmer CHN analyzer, model no. 2400. Monitoring of reactions and checking of purity of the final products were carried out by thin layer chromatography (TLC) using silica gel precoated aluminium sheets (60 F254, Merck), The developing solvent system was chloroform/methanol (8:2) and the spots were detected with ultraviolet light (UV) at 365 and 254 nm. (RS)-2-(3,4,5-tri-chlorophenoxypropionic acid, silvex, was obtained from Acros (Geel, Belgium), aldehydes, acetophenones and thioglycolic acid were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Antimicrobial activity was done at the research centre, college of Pharmacy, King Saud University, Saudi Arabia.

*2-(2,4,5-Trichlorophenoxy)propanehydrazide (**1**)*

A solution of 2-(2,4,5-Trichlorophenoxy)propanoic acid (10 mmol, 2.69 g) and ethanol (20 ml) containing few drops of sulphuric acid was refluxed for 4 h. The reaction mixture was cooled and excess alcohol was removed under reduced pressure. The remaining residue was poured onto a 5 % aqueous sodium bicarbonate solution (25 ml). Then the aqueous layer was extracted using diethyl ether. The organic layer extract was dried over anhydrous magnesium sulphate followed by evaporation of the ether solvent under reduced pressure. The obtained oily product of the crude ester and an excess of hydrazine hydrate (30 mmol) in ethanol (20 ml) was refluxed for 3 h and cooled. The solid was separated by filtration and recrystallised from ethanol to afford 2-(2,4,5-trichlorophenoxy)propanehydrazide **1** as colourless fine needles. Yield 83 %; m.p. 210-211 °C; IR, (KBr, cm^{-1}): 3318, 3264 (NH, NH_2), 3,031(CH-arom.), 2985 (CH-aliph.), 1662 (C=O). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.45 [d, 3H, CH_3], 4.25 [s, 2H, NH_2, D_2O exchangeable], 4.72 [q, 1H, O–CH–CO], 7.15 [s, 1H, trichlorophenyl-H6], 7.61 [s, 1H, trichlorophenyl-H3], 9.18 [s, 1H, CONH–, D_2O exchangeable]. Anal. Calcd. for $C_9H_9Cl_3N_2O_2$: C, 38.12; H, 3.20; N, 9.88. Found: C, 38.35; H, 3.31; N, 9.72.

*2-(2,4,5-Trichlorophenoxy)propanehydrazide hydrazones (**2**–**11**)*

A mixture of **1** (10 mmol, 2.83 g) and a corresponding carbonyl compound (20 mmol) in ethanol (50 ml) containing a catalytic amount of glacial acetic acid (1 ml) was refluxed for 2 h. The mixture was cooled; the solid was separated by filtration and recrystallized from ethanol to give the corresponding hydrazide–hydrazones **2**–**11**.

*2-(2,4,5-Trichlorophenoxy)-N′-(3-nitrobenzylidene)propanehydrazide (**2**)*

Yield 95 %; m.p. 230–231 °C; IR (KBr, cm^{-1}): 3174 (NH), 3092 (CH-arom.), 2938 (CH-aliph.), 1666 (C=O), 1609 (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.6 [d, 3H, CH_3], 5.07 [q, 1H, –O–CH–CO], 7.24 [s, 1H, trichlorophenyl-H6], 7.72 [t, 1H, Ar–H5], 7.85 [s, 1H, trichlorophenyl-H3], 8.12 [d, 1H, Ar–H6], 8.25, [s, 1H, CH=N], 8.43 [d, 1H, Ar–H4], 8.52 [s, 1H, Ar–H2], 11.92 [s, 1H, CONH, D_2O exchangeable]. ^{13}C-NMR (DMSO-d_6, δ, ppm): 17.2 (CH_3), 72.1 (O–C–), 152.29, 148.1, 146.0, 142.2, 135.6, 132.8, 130.8, 130.1, 124.2, 123.1, 121.1, 115.7 (aromatic carbons), 166.49 (CH=N), 170.7 (C=O). MS (*m*/*z*): 417 [M^++2] (8.5), 415 [M^+] (8.7), 41 (100). Anal. Calcd. for $C_{16}H_{12}Cl_3N_3O4$: C, 46.12; H, 2.90; N, 10.09. Found: C, 46.37; H, 3.11; N, 10.23.

*2-(2,4,5-Trichlorophenoxy)-N′-((thiophen-2-yl)methylene)propanehydrazide (**3**)*

Yield 78 %; m.p. 210–211 °C; IR (KBr, cm^{-1}): 3168 (NH), 3084 (CH-arom.), 2953 (CH-aliph.), 1672 (C=O), 1612 (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.56 [d, 3H, CH_3], 5.00 [q, 1H, –O–CH–CO], 7.15 [t, 1H, Ar–H4], 7.31 [s, 1H, trichlorophenyl-H6], 7.46 [d, 1H, Ar–H5], 7.65 [d, 1H, Ar–H3], 7.84 [s, 1H, trichlorophenyl-H3], 8.22 [s, 1H, CH=N], 11.60 [s, 1H, CONH, D_2O exchangeable]. ^{13}C-NMR ($CDCl_3$, δ, ppm): 17.1 (CH_3), 71.8 (O–C–), 115.6, 121.5, 123.0, 127.8, 128.7, 130.2, 138.4, 139.5, 143.5, 152.3 (aromatic carbons), 165.9 (C=N), 170.0 (C=O). MS (m/z): 377 [M^++2] (3.3), 375 [M^+] (3.7), 41 (100). Anal. Calcd. for $C_{14}H_{11}Cl_3N_2O_2S$: C, 44.52; H, 2.94; N, 7.42; S, 8.49. Found: C, 44.79; H, 3.13; N, 7.58; S, 8.65.

*2-(2,4,5-Trichlorophenoxy)-N′-((5-methylthiophen-2-yl)methylene)propanehydrazide (**4**)*

Yield 75 % m.p. 188–189 °C; IR (KBr, cm^{-1}): 3188 (NH), 3094 (CH-arom.), 2947 (CH-aliph.), 1662 (C=O), 1604 (C=N). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.55 [d, 3H, CH_3], 2.46 [s, 3H, Ar–CH_3], 4.99 [q, 1H, –O–CH–CO], 6.81 [d, 1H, Ar–H4], 7.26 [d, 1H, Ar–H3], 7.31 [s, 1H, trichlorophenyl-H6], 7.84 [s, 1H, trichlorophenyl-H3], 8.10 [s, 1H, CH=N], 11.53 [s, 1H, CONH, D_2O exchangeable]. ^{13}C-NMR ($CDCl_3$, δ, ppm): 15.2 (Ar–CH_3), 17.0 (CH_3), 71.9 (O–CH–), 115.6, 121.6, 123.0, 126.2, 130.2, 131.1, 136.2, 139.5, 142.6, 152.3 (aromatic carbons), 165.7 (C=N), 169.8 (C=O). MS (*m*/*z*): 391 [M^++2] (6.8), 389 [M^+] (7.2), 105 (100). Anal. Calcd. for $C_{15}H_{13}Cl_3N_2O_2S$: C, 45.99; H, 3.35; N, 7.15; S, 8.19. Found: C, 45.72; H, 3.51; N, 7.01; S, 7.92.

*2-(2,4,5-Trichlorophenoxy)-N′-((5-methylfuran-2-yl)methylene)propanehydrazide (**5**)*

Yield 73 %; m.p. 207–208 °C; IR, (KBr, cm^{-1}): 3216 (NH), 3053(CH-arom.), 2971 (CH-aliph.), 1667 (C=O), 1624 (C=N). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.73 [d, 3H, CH_3], 2.36 [s, 3H, Ar–CH_3], 4.84 [q, 1H, –O–CH–CO], 6.14 [d, 1H, Ar–H4], 6.7 [d, 1H, Ar–H3], 7.13 [s, 1H, trichlorophenyl-H6], 7.52 [s, 1H, trichlorophenyl-H3], 8.20 [s, 1H, CH=N], 11.27 [s, 1H, CONH, D_2O exchangeable]. ^{13}C-NMR ($CDCl_3$, δ, ppm): 15.3 (Ar–CH_3), 17.9 (CH_3), 58.4 (O–CH–), 108.5, 116.9, 117.9, 122.7, 126.4, 131.0, 131.9, 139.8, 147.0, 151.1 (aromatic carbons), 156.1 (CH=N), 167.6 (C=O). MS (*m*/*z*): 376 [M^++2] (4.2), 374 [M^+] (3.9), 52 (100). Anal. Calcd. for $C_{15}H_{13}Cl_3N_2O_3$: C, 47.96; H, 3.49; N, 7.46. Found: C, 48.15; H, 3.63; N, 7.58.

*2-(2,4,5-Trichlorophenoxy)-N′-((pyridin-3-yl)methylene)propanehydrazide (**6**)*

Yield 82 %; m.p. 192–193 °C; IR, (KBr, cm^{-1}): 3204 (NH), 3062 (CH-arom.), 2994 (CH-aliph.), 1670 (C=O), 1609 (C=N). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.75 [d, 3H, CH_3], 4.9 [q, 1H, –O–CH–CO], 7.10 [s, 1H, trichlorophenyl-H6], 7.36 [t, 1H, Ar–H5], 7.92 [s, 1H, trichlorophenyl-H3], 8.20 [d, 1H, Ar–H6], 8.28 [s, 1H, CH=N], 8.65, [d, 1H, Ar–H4], 8.82 [d, 1H, Ar–H2], 10.62 [s, 1H, CONH, D_2O exchangeable]. ^{13}C-NMR ($CDCl_3$, δ, ppm): 17.8 (CH_3), 72.7 (O–CH–),116.2, 122.8, 126.6, 129.2, 131.3, 133.7, 142.8, 146.5, 148.9, 151.1, 152.6 (aromatic carbons), 166.9 (CH=N), 172.5 (C=O). MS (*m/z*): 373 [M^++2] (6.7), 371 [M^+] (6.1), 148 (100). Anal. Calcd. for $C_{15}H_{12}Cl_3N_3O_2$: C, 48.35; H, 3.25; N, 11.28. Found: C, 48.20; H, 3.39; N, 11.01.

*2-(2,4,5-Trichlorophenoxy)-N′-((pyridin-4-yl)methylene)propanehydrazide (**7**)*

Yield 85 % m.p. 163–164 °C; IR, (KBr, cm−1): 3286 (NH), 3100 (CH-arom.), 2940 (CH-aliph.), 1687 (C=O), 1600 (C=N). 1H-NMR (CDCl3, δ, ppm): 1.75 [d, 3H, CH3], 4.91 [q, 1H, –O–CH–CO], 7.10 [s, 1H, trichlorophenyl-H6], 7.63 [d, 2H, Ar–H2, Ar–H6], 7.73 [s, 1H, trichlorophenyl-H3], 8.25 [s, 1H, CH=N], 8.68 [d, 2H, Ar–H3, Ar–H5], 10.7 [s, 1H, CONH, D_2O exchangeable]. 13C-NMR (DMSO-d_6, δ, ppm): 17.8 (CH_3), 72.8 (O–CH–), 116.3, 121.0, 126.7, 131.2, 140.8, 143.1, 146.9, 150.0, 152.5 (aromatic carbons), 167.1 (CH=N), 172.7 (C=O). MS (*m/z*): 373 [M^++2] (7.3), 371 [M+] (6.8), 43 (100). Anal. Calcd. for $C_{15}H_{12}C_{l3}N_3O_2$: C, 48.35; H, 3.25; N, 11.28. Found: C, 48.02; H, 3.39; N, 11.15.

*2-(2,4,5-Trichlorophenoxy)-N′-(1-p-tolylethylidene)propanehydrazide (**8**)*

Yield 93 %; m.p. 196–197 °C; IR, (KBr, cm^{-1}): 3201 (NH), 3037 (CH-arom.), 2934 (CH-aliph.), 1664 (C=O), 1610 (C=N). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.77 [d, 3H, CH_3], 2.25 [s, 3H, *p*-tolyl-CH_3], 2.31 [s, 3H, ethylidene-CH_3], 4.98 [q, 1H, –O–CH–CO], 7.11 [s, 1H, trichlorophenyl-H6], 7.22 [d, 2H, Ar–H3, Ar–H5], 7.55 [s, 1H, trichlorophenyl-H3], 7.76 [d, 2H, Ar–H2, Ar–H6], 9.58 [s, 1H, CONH, D_2O exchangeable]. ^{13}C-NMR ($CDCl_3$, δ, ppm): 12.8 (Ar–CH_3), 17.8 (CH_3), 21.3 (CH_3), 73.0 (O–CH–), 116.2, 122.4, 126.1, 129.2, 130.3, 131.9, 134.5, 140.0, 150.8, 154.0 (aromatic carbons), 166.0 (C=N), 172.0 (C=O). MS (*m/z*): 398 [M^++2] (3.2), 400 [M^+] (3.5). Anal. Calcd. for $C_{18}H_{17}Cl_3N_2O_2$: C, 54.09; H, 4.29; N, 7.01. Found: C, 53.82; H, 4.03; N, 7.22.

*2-(2,4,5-Trichlorophenoxy)-N′-(1-(4-chlorophenyl)ethylidene)propanehydrazide (**9**)*

Yield 95 % m.p. 198–199 °C; IR, (KBr, cm^{-1}): 3174 (NH), 3033 (CH-arom.), 2986 (CH-aliph.), 1674 (C=O), 1613 (C=N).^{1}H-NMR ($CDCl_3$, δ, ppm): 1.67 [d, 3H, CH_3], 2.31 [s, 3H, CH_3–C=], 4.98 [q, 1H, –O–CH–CO], 7.11 [s, 1H, trichlorophenyl-H6], 7.28 [s, 1H, trichlorophenyl-H3], 7.39 [d, 2H, Ar–H3, Ar–H5], 7.81 [d, 2H, Ar–H2, Ar–H6], 9.63 [s, 1H, CONH, D_2O exchangeable]. ^{13}C-NMR ($CDCl_3$, δ, ppm): 13.2 (CH_3), 18.2 (CH_3), 73.4 (O–CH–), 116.2, 122.4, 127.4, 128.6, 129.7, 131.1, 132.0, 135.8, 136.1, 152.6 (aromatic carbons), 166.1 (C=N), 171.8 (C=O). MS (*m/z*): 420 [M^++2] (10.7), 418 [M^+] (9.1). Anal. Calcd. for $C_{17}H_{14}Cl_4N_2O_2$: C, 48.60; H, 3.36; N, 6.67. Found: C, 48.79; H, 3.18; N, 6.51.

*2-(2,4,5-Trichlorophenoxy)-N′-(2-oxoindolin-3-ylidene)propanehydrazide (**10**)*

Yield 91 %; m.p. 208–209 °C; IR, (KBr, cm^{-1}): 3183 (NH), 3073 (CH-arom.), 2934 (CH-aliph.), 1709 (C=O), 1679 (C=O), 1622 (C=N). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.78 [d, 3H, CH_3], 4.97 [q, 1H, –O–CH–CO], 6.99 [d, 1H, isatin-H4], 7.1 [s, 1H, trichlorophenyl-H6], 7.14 [t, 1H, isatin-H5], 7.37 [t, 1H, isatin-H6], 7.54 [s, 1H, trichlorophenyl-H3], 7.82 [d, 1H, isatin-H7], 7.96 [s, 1H, isatin-NH, D_2O exchangeable], 8.1 [s, 1H, CONH, D_2O exchangeable]. ^{13}C-NMR ($CDCl_3$, δ, ppm): 18.52 (CH_3), 58.49 O–CH–), 110.8, 116.9, 122.6, 123.4, 123.6, 126.3, 131.4, 132.2, 138.8, 141.0, 151.3 (aromatic carbons), 162.3 (C=O), 168.6 (C=O). Mass *m/z* [%]: 412 [M^++2] (5.3), 410 [M^+] (5.1), 43 (100). Anal. Calcd. for $C_{17}H_{12}Cl_3N_3O_3$: C, 49.48; H, 2.93; N, 10.18. Found: C, 49.21; H, 2.80; N, 10.02.

*2-(2,4,5-Trichlorophenoxy)-N′-(2-oxo-1-(prop-2-ynyl)indolin-3-ylidene)propanehydrazide (**11**)*

Yield 85 % m.p. 182–183 °C; IR, (KBr, cm^{-1}): 3209 (NH), 3097 (CH-arom.), 2938 (CH-aliph.), 2237 (C≡C), 1713 (C=O), 1677 (C=O) 1614 (C=N). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.65 [d, 3H, CH_3], 2.18 [s, 1H, ≡CH], 4.49 [s, 2H, ≡C–CH_2–N], 4.94 [q, 1H, –O–CH–CO], 7.0 [d, 1H, isatin-H4], 7.1 [s, 1H, trichlorophenyl-H6], 7.17 [t, 1H, isatin-H5], 7.44 [t, 1H, isatin-H6], 7.54 [s, 1H, trichlorophenyl-H3], 7.84 [d, 1H, isatin-H7], 8.0 [s, 1H, CONH, D_2O exchangeable]. ^{13}C-NMR ($CDCl_3$, δ, ppm): 18.5 (CH_3), 29.4 (propynyl-C1), 63.4 (propynyl-C3), 71.3 (O–CH–), 86.8 (propynyl-C2), 110.2, 117.5, 119.4, 120.9, 123.7, 126.5, 131.2, 131.9, 138.8, 142.5, 151.4 (aromatic carbons), 160.2 (C=O), 168.6 (C=O). Mass *m/z* [%]: 451 [M^++2] (15.0), 449 [M^+] (13.5), 61 (100.0). Anal. Calcd.

for $C_{20}H_{14}Cl_3N_3O_3$: C, 53.30; H, 3.13; N, 9.32. Found: C, 53.58; H, 3.01; N, 9.51.

*2-(2,4,5-Trichlorophenoxy)-N-(4-oxo-2-substitutedthiazolidin-3-yl)propanamide (**12–21**)*

To a well-stirred suspension of the appropriate hydrazone derivatives (**2**–**11**), (10 mmol) in dry benzene (50 ml), mercaptoacetic acid (4.2 ml, 60 mmol) in dry benzene (10 ml) was added. The reaction mixture was refluxed for 15-18 h and the solvent was evaporated under reduced pressure. The residue was triturated with boiling water (100 ml), left overnight, filtered off, washed with water, dried and crystallised from the proper solvent affording the title compounds (**12**–**21**).

*2-(2,4,5-Trichlorophenoxy)-N-(2-(3-nitrophenyl)-4-oxothiazolidin-3-yl)propanamide (**12**)*

Yield 75 % m.p. 182–183 °C; IR (KBr, cm^{-1}): 3173 (NH), 3085 (CH-arom.), 2985 (CH-aliph.), 1728 (C=O thiazolidinone), 1669 (C=O amide). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.6 [d, 3H, CH_3], 3.34 [s, 2H, thiazolidinone-H3], 5.08 [q, 1H, –O–CH–CO], 5.85 [s, 1H, thiazolidinone-H5], 7.26 [s, 1H, trichlorophenyl-H6], 7.34 [s, 1H, trichlorophenyl-H3], 7.74 [dd, 1H, Ar–H5], 7.82 [s, 1H, Ar–H6], 8.12 [d, 1H, Ar–H4], 8.16 [s, 1H, Ar–H2], 11.9 [s, 1H, CONH, D_2O exchangeable]. ^{13}C-NMR (DMSO-d_6, δ, ppm): 18.09 (CH_3), 41.66 (thiazolidinone N–CH–S), 66.32 (thiazolidinone CH_2), 74.46 (O–CH–), 115.85, 121.10, 123.10, 124.44, 130.1, 130.88, 133.34, 135.78, 142.30, 146.0, 148.20, 152.70 (aromatic carbons), 166.48 (thiazolidinone C=O), 170.78 (C=O). Anal. Calcd. for $C_{18}H_{14}Cl_3N_3O_5S$: C, 44.05; H, 2.88; N, 8.56. Found: C, 44.31; H, 3.02; N, 8.35.

*2-(2,4,5-Trichlorophenoxy)-N-(4-oxo-2-(thiophen-2-yl)thiazolidin-3-yl)propanamide (**13**)*

Yield 78 % m.p. 123–124 °C; IR, (KBr, cm^{-1}): 3164 (NH), 3046 (CH-arom.), 2982 (CH-aliph.), 1721 (C=O thiazolidinone), 1677 (C=O amide). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.43 [d, 3H, CH_3], 3.24 [s, 2H, thiazolidinone-H3], 4.87 [q, 1H, –O–CH–CO], 6.07 [s, 1H, thiazolidinone-H5], 6.98–7.00 [m, 2H, Ar–H3 and Ar–H4], 7.21 [d, 1H, Ar–H5], 7.31 [s, 1H, trichlorophenyl-H6], 7.83 [s, 1H, trichlorophenyl-H3], 12.60 [s, 1H, CONH, D_2O exchangeable]. ^{13}C-NMR (DMSO-d_6, δ, ppm): 18.16 (CH_3), 40.55 (thiazolidinone N–CH–S), 57.29 (thiazolidinone CH_2), 73.79 (O–CH–),116.3, 121.8, 123.7, 126.7, 128.4, 128.9, 130.2, 130.6, 141.4, 152.2 (aromatic carbons), 168.02 (thiazolidinone C=O), 172.04 (C=O). Mass *m/z* [%]: 451 [M^++2] (4.5), 449 [M^+] (4.3), 45 (100.0). Anal. Calcd. for $C_{16}H_{13}Cl_3N_2O_3S_2$: C, 42.54; H, 2.90; N, 6.20. Found: C, 42.15; H, 2.78; N, 6.46.

*2-(2,4,5-Trichlorophenoxy)-N-(2-(5-methylthiophen-2-yl)-4-oxothiazolidin-3-yl)propanamide (**14**)*

Yield 78 % m.p. 135–136 °C; IR, (KBr, cm^{-1}): 3175 (NH), 3062 (CH-arom.), 2925 (CH-aliph.), 1722 (C=O thiazolidinone), 1685 (C=O amide).^{1}H-NMR (DMSO-d_6, δ, ppm): 1.47 [d, 3H, CH_3], 2.25 [s, 3H, Ar–CH_3], 3.49 [s, 2H, thiazolidinone-H3], 4.82 [q, 1H, –O–CH–CO], 5.96 [s, 1H, thiazolidinone-H5], 6.65 [d, 1H, Ar–H4], 6.90 [d, 1H, Ar–H3], 7.21 [s, 1H, trichlorophenyl-H6], 7.83 [s, 1H, trichlorophenyl-H3], 12.67 [s, 1H, CONH, D_2O exchangeable]. ^{13}C-NMR (DMSO-d_6, δ, ppm): 15.25 (Ar–CH_3), 18.75 (CH_3), 40.54 (thiazolidinone N–CH-S), 57.27 (thiazolidinone CH_2), 73.24 (O–CH–), 116.25, 121.59, 123.60, 125.04, 126.60, 129.04, 130.2, 130.6, 141.8, 152.1 (aromatic carbons), 170.46 (thiazolidinone C=O), 172.04 (C=O). Mass *m/z* [%]: 465 [M^++2] (2.4), 463 [M^+] (2.2), 118 (100.0). Anal. Calcd. for $C_{17}H_{15}Cl_3N_2O_3S_2$: C, 43.83; H, 3.25; N, 6.01. Found: C, 43.61; H, 3.40; N, 6.25.

*2-(2,4,5-Trichlorophenoxy)-N-(2-(5-methylfuran-2-yl)-4-oxothiazolidin-3-yl)propanamide (**15**)*

Yield 73 %; m.p. 150–151 °C; IR, (KBr, cm^{-1}): 3213 (NH), 3100 (CH-arom.), 2907 (CH-aliph.), 1708 (C=O thiazolidinone), 1668 (C=O amide).^{1}H-NMR (DMSO-d_6, δ, ppm): 1.49 (d, 3H, CH_3), 2.26 (s, 3H, Ar–CH_3), 3.48 (s, 2H, thiazolidinone-H3), 4.92 (q, 1H, –O–CH–CO), 5.77 (s, 1H, thiazolidinone-H5), 6.03 (d, 1H, Ar–H4), 6.40 (d, 1H, Ar–H3), 7.30 (s, 1H, trichlorophenyl-H6), 7.82 (s, 1H, trichlorophenyl-H3), 11.50 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6, δ, ppm): 13.43 (Ar–CH_3), 18.65 (CH_3), 38.99 (thiazolidinone N–CH-S), 53.01 (thiazolidinone CH_2), 74.58 (O–CH–), 108.58, 116.48, 122.08, 128.28, 130.86, 134.66, 137.94, 147.47, 152.31, 154.82 (aromatic carbons), 165.88 (thiazolidinone C=O), 170.11 (C=O). Mass *m/z* [%]: 449 [M^++2] (4.2), 447 [M^+] (4.4), 62 (100.0). Anal. Calcd. for $C_{17}H_{15}Cl_3N_2O_4S$: C, 45.40; H, 3.36; N, 6.23. Found: C, 45.18; H, 3.11; N, 6.52.

*2-(2,4,5-Trichlorophenoxy)-N-(4-oxo-2-(pyridin-3-yl)thiazolidin-3-yl)propanamide (**16**)*

Yield 73 % m.p. 212–213 °C; IR, (KBr, cm^{-1}): 3190 (NH), 3061 (CH-arom.), 2989 (CH-aliph.), 1704 (C=O thiazolidinone), 1680 (C=O amide).^{1}H-NMR (DMSO-d_6, δ, ppm): 1.40 [d, 3H, CH_3], 3.63 [s, 2H, thiazolidinone-H3], 4.84 [q, 1H, –O–CH–CO], 5.83 [s, 1H, thiazolidinone-H5], 7.14 [s, 1H, trichlorophenyl-H6], 7.41 [dd, 1H, Ar–H5],

7.79 [s, 1H, trichlorophenyl-H3], 7.89 [d, 1H, Ar–H4], 8.55 [d, 1H, Ar–H6], 8.62 [s, 1H, Ar–H2], 10.64 [s, 1H, CONH, D_2O exchangeable]. ^{13}C-NMR (DMSO-d_6, δ, ppm): 18.55 (CH_3), 29.12 (thiazolidinone N–CH–S), 59.49 (thiazolidinone CH_2), 73.66 (O–CH–), 116.42, 121.83, 123.5, 123.71, 130.27, 130.69, 133.73, 135.67, 149.06, 150.17, 151.98 (aromatic carbons), 168.50 (thiazolidinone C=O), 169.01 (C=O). Mass *m/z* [%]: 446 [M^++2] (5.2), 444 [M^+] (5.0), 176 (100). Anal. Calcd. for $C_{17}H_{14}Cl_3N_3O_3S$: C, 45.71; H, 3.16; N, 9.41. Found: C, 46.04; H, 3.03; N, 9.18.

*2-(2,4,5-Trichlorophenoxy)-N-(4-oxo-2-(pyridin-4-yl)thiazolidin-3-yl)propanamide (**17**)*

Yield 73 % m.p. 155–156 °C; IR, (KBr, cm^{-1}): 3191 (NH), 3100 (CH-arom.), 2960 (CH-aliph.), 1705 (C=O thiazolidinone), 1665 (C=O amide). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.50 (d, 3H, CH_3), 3.51 (s, 2H, thiazolidinone-H3), 5.10 (q, 1H, –O–CH–CO), 5.85 (s, 1H, thiazolidinone-H5), 7.25 (s, 1H, trichlorophenyl-H6), 7.62 (d, 2H, Ar–H3, Ar–H5), 7.80 (s, 1H, trichlorophenyl-H3), 8.64 (d, 2H, Ar–H2 and Ar–H6), 12.07 (s, 1H, CONH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, δ, ppm): 18.19 (CH_3), 29.12 (thiazolidinone N–CH–S), 72.09 (thiazolidinone CH_2), 74.33 (O–CH–), 115.83, 121.02, 123.64, 130.78, 140.95, 142.01, 146.04, 150.11, 152.69 (aromatic carbons), 166.61 (thiazolidinone C=O), 170.95 (C=O). Mass *m/z* [%]: 446 [M^++2] (6.9), 444 [M^+] (6.4), 197 (100). Anal. Calcd. for $C_{17}H_{14}Cl_3N_3O_3S$: C, 45.71; H, 3.16; N, 9.41. Found: C, 45.95; H, 3.38; N, 9.23.

*2-(2,4,5-Trichlorophenoxy)-N-(2-methyl-4-oxo-2-p-tolylthiazolidin-3-yl)propanamide (**18**)*

Yield 80 % m.p. 154–155 °C; IR, (KBr, cm^{-1}): 3175 (NH), 3031 (CH-arom.), 2973 (CH-aliph.), 1692 (C=O thiazolidinone), 1667 (C=O amide). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.50 [d, 3H, CH_3], 2.26 [s, 3H, thiazolidinone-5-CH_3], 2.36 [s, 3H, Ar–4-CH_3], 3.35 [s, 2H, thiazolidinone-H3], 4.94 [q, 1H, –O–CH–CO], 7.20 [d, 2H, Ar–H3, Ar–H5], 7.31 [s, 1H, trichlorophenyl-H6], 7.60 [d, 2H, Ar–H2 and Ar–H6], 7.83 [s, 1H, trichlorophenyl-H3], 10.37 [s, 1H, CONH, D_2O exchangeable]. ^{13}C-NMR (DMSO-d_6, δ, ppm): 13.60 (Ar–CH_3), 18.60 (CH_3), 20.80 (CH_3), 25.03 (thiazolidinone N–CH–S), 72.34 (thiazolidinone CH_2), 73.93 O–CH–), 115.60, 123.59, 126.34, 128.89, 130.05, 130.87, 134.98, 138.90, 149.47, 152.80 (aromatic carbons), 167.0 (thiazolidinone C=O), 171.28 (C=O). Mass *m/z* [%]: 474 [M^++2] (5.7), 472 [M^+] (5.4), 62 (100). Anal. Calcd. for $C_{20}H_{19}Cl_3N_2O_3S$: C, 50.70; H, 4.04; N, 5.91. Found: C, 50.52; H, 4.25; N, 5.65.

*2-(2,4,5-Trichlorophenoxy)-N-(2-(4-chlorophenyl)-2-methyl-4-oxothiazolidin-3-yl)propanamide (**19**)*

Yield 84 % m.p. 195–196 °C; IR, (KBr, cm^{-1}): 3174 (NH), 3034 (CH-arom.), 2985 (CH-aliph.), 1693 (C=O thiazolidinone), 1668 (C=O amide). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.59 [d, 3H, CH_3], 2.28 [s, 3H, thiazolidinone-5-CH_3], 3.34 [s, 2H, thiazolidinone-H3], 5.25 [q, 1H, –O–CH–CO], 7.17 [s, 1H, trichlorophenyl-H6], 7.44 [d, 2H, Ar–H3, Ar–H5], 7.49 [d, 2H, Ar–H2 and Ar–H6], 7.77 [s, 1H, trichlorophenyl-H3], 10.99 [s, 1H, CONH, D_2O exchangeable]. ^{13}C-NMR (DMSO-d_6, δ, ppm): 17.40 (CH_3), 18.32 (CH_3), 34.50 (thiazolidinone N–CH–S), 66.31 (thiazolidinone CH_2), 73.62 (O–CH–), 115.70, 122.95, 127.90, 128.28, 130.04, 130.73, 133.97, 136.55, 148.19, 152.50 (aromatic carbons), 166.5 (thiazolidinone C=O), 171.41 (C=O). Mass *m/z* [%]: 493 [M^++2] (6.9), 491 [M^+] (7.8), 47 (100). Anal. Calcd. for $C_{19}H_{16}Cl_4N_2O_3S$: C, 46.17; H, 3.26; N, 5.67. Found: C, 46.42; H, 3.04; N, 5.46.

*2-(2,4,5-Trichlorophenoxy)-N-(2,4′-dioxo-1,2-dihydro-3′H-spiro[indole-3,2′-[1,3]thiazolidin]-3′-yl)propanamide (**20**)*

Yield 78 % m.p. 216–218 °C; IR, (KBr, cm^{-1}): 3226 (NH), 3096 (CH-arom.), 2938 (CH-aliph.), 1722 (C=O isatin), 1711 (C=O thiazolidinone), 1651 (C=O amide). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.61 [d, 3H, CH_3], 3.35 [s, 2H, thiazolidinone-H3], 5.43 [q, 1H, –O–CH–CO], 6.93 [d, 1H, Ar–H7], 7.09 [t, 1H, Ar–H5], 7.37 [t, 1H, Ar–H6], 7.39 [s, 1H, trichlorophenyl-H6], 7.61 [d, 1H, Ar–H4], 7.88 [s, 1H, trichlorophenyl-H3], 11.24 [s, 1H, CONH, D_2O exchangeable], 13.55 [s, 1H, indolinone-NH, D_2O exchangeable]. ^{13}C-NMR (DMSO-d_6, δ, ppm): 17.7 (CH_3), 63.01 (thiazolidinone CH_2), 73.02 (thiazolidinone-C2), 75.1 (O–CH–), 111.1, 116.9, 119.6, 121.0, 122.6, 128.3, 130.4, 130.9, 131.8, 132.0, 133.0, 142.7 (aromatic carbons), 162.4 (two exocyclic CO), 167.5 (C=O). Mass *m/z* [%]: 486 [M^++2] (8.9), 484 [M^+] (9.1), 61 (100). Anal. Calcd. for $C_{19}H_{14}Cl_3N_3O_4S$: C, 46.88; H, 2.90; N, 8.63. Found: C, 47.03; H, 2.71; N, 8.41.

*2-(2,4,5-Trichlorophenoxy)-N-(2,4′-dioxo-1-(prop-2-ynyl)-1,2-dihydro-3′H-spiro[indole-3,2′-[1,3]thiazolidin]-3′-yl)propanamide (**21**)*

Yield 76 % m.p. 177–178 °C; IR, (KBr, cm^{-1}): 3205 (NH), 3082 (CH-arom.), 2937 (CH-aliph.), 2235 (C≡C), 1728 (CO isatin) 1712 (C=O thiazolidinone), 1688 (C=O amide). ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.55 (d, 3H, CH_3), 2.15 (s, 1H, ≡CH), 3.48 (s, 2H, propynyl-CH_2), 3.71 (s, 2H, thiazolidinone-H3), 4.83 (q, 1H, –O–CH–CO), 7.09 (d, 1H, Ar–H7), 7.18 (s, 1H, trichlorophenyl-H6), 7.2 (t, 1H,

Ar–H5), 7.32 (t, 1H, Ar–H6), 7.57 (s, 1H, trichlorophenyl-H3), 7.84 (d, 1H, Ar–H4), 11.50 (s, 1H, CONH). Mass *m/z* [%]: 524 [M^++2] (10.7), 522 [M^+] (11.0), 62 (100). Anal. Calcd. for $C_{22}H_{16}Cl_3N_3O_4S$: C, 50.35; H, 3.07; N, 8.01. Found: C, 50.61; H, 3.25; N, 7.83.

Antimicrobial evaluations

Compounds (**2**–**21**) were evaluated for their in vitro antimicrobial activity against Gram positive bacteria: *S. aureus* (ATCC 25923), *B. subtilis* (ATCC 10400), Gram negative *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27855), *Shig. Sonnei* (ATCC 11060) and fungal strains: *C. albicans* (ATCC 10231). Antimicrobial activity was assessed by serial twofold dilution technique (Madhukar *et al.*, 2009). Ciprofloxacin was used as a standard drug for antibacterial activity and miconazole was used as a standard drug for antifungal activity. All the compounds were dissolved in dimethyl sulfoxide to give a concentration of 100 μg ml^{-1}. Twofold dilutions of test and standard compounds were prepared in double strength nutrient broth (bacteria) or Sabouraud dextrose broth (fungi). The stock solution was serially diluted to give concentrations of 100–6.25 μg ml^{-1} in nutrient broth. The inoculum size was approximately 106 colony forming units (CFU)/ml. The tubes were incubated at 37 ± 1 °C for 24 h (bacteria) and 25 °C for 7 days (*fungi*). After that, the inoculated culture tubes were macroscopically examined for turbidity. The culture tube showing turbidity (lower concentration) and the culture tube showing no turbidity (higher concentration) gave the minimum inhibitory concentration (MIC) for the compound.

Antimycobacterium tuberculosis activity

Preliminary screening of compounds **2**–**21** for their in vitro antituberculosis was conducted at 100 μg ml^{-1} against *M. tuberculosis* H37Rv in BACTEC 12B medium using a broth microdilution assay, the MABA (Collins and Franzblau, 1997). Rifampin was used as the standard in the tests. None of the compounds were considered for further evaluation as they had mycobacterial inhibitions less than 90 % at more than 200 μg ml^{-1} (Küçükgüze *et al.*, 2006).

Docking procedure

All molecular modelling studies were performed on PC windows Vista Home Premium Intel(R) Core(TM)2 Duo, 1.83 GHz using Dock6.4 (Lang *et al.*, 2009). All compounds were generated in the protonation state under physiological condition. The coordinates of UDP-*N*-acetylmuramate dehydrogenase (*MURB*) enzyme X-ray structure, complexed with its co-crystallised ligand naphthyl tetronic acid inhibitor, (2Q85) was taken from the Protein Data Bank (Mansour *et al.*, 2007). The co-crystallised ligand was docked in its original protein structure. Docking was performed with default settings to obtain a population of possible conformations and orientations for the inhibitors at the binding site. A 10-Å sphere around the centre of the binding pocket was defined as binding pocket for the docking runs. All torsion angles in each compound were allowed to rotate freely.

Conclusion

It was concluded that thiazolidinone analogues (**12**–**21**) are selectively active against the tested Gram positive bacteria. Compounds **13** and **17** were found equipotent with the reference drug, chloramphenicol, with MIC value 6.25 μg ml^{-1}. A good correlation was found between the in silico generated model and the reported X-ray structure with similar hydrogen bonding and orientation inside the binding site. On the other hand, the hydrazide-hydrazone derivatives (**2**–**11**) showed no antimicrobial activity.

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