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RESEARCH ARTICLE

Synthesis and biological evaluation of some novel urea and thiourea derivatives of isoxazolo[4,5-*d*]pyridazine and structurally related thiazolo[4,5-*d*]pyridazine as antimicrobial agents

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Abstract This study reports the synthesis of some novel isoxazolo[4,5-*d*]pyridazines and structurally related thiazolo[4,5-*d*]pyridazines, and their biological evaluation as antimicrobial agents. The proposed compounds were designed to contain pharmacophores such as urea, thiourea, sulfonylurea (thiourea) and some derived functionalities that are believed to contribute to the anticipated biological activities. The results revealed that 25 compounds displayed broad spectrum of antibacterial activity, with greater inhibitory effect on the growth of the tested Gram positive strains compared to Gram negative ones. Moreover, 14 compounds were able to produce appreciable growth inhibitory activity against *Candida albicans* fungus when compared to Clotrimazole. Most of the tested isoxazolo[4,5-*d*]pyridazines displayed better antimicrobial profile than their corresponding thiazolo[4,5-*d*]pyridazine congeners. Four compounds namely, *p*-(3,7-dimethyl-4-oxo-4*H*-isoxazolo[4,5-*d*]pyridazine-5-yl)benzenesulfonylthioureas (**11c–d**), 3-substituted-2-[*p*-(3,7-dimethyl-4-oxo-4*H*-isoxazolo[4,5-*d*]pyridazine-5-yl)-benzene-sufonylimino]-4-oxothiazolidines (**13d**) and *p*-(2,7-dimethyl-4-oxo-4*H*-thiazolo[4,5-*d*]pyridazin-5-yl)benzenesulfonylthiourea (**24c**) were found to be most active antimicrobial members in present study.

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Introduction

The past few years have witnessed an obvious reduction in the mortality caused by infectious diseases. However, the emergence of Gram-positive and Gram-negative pathogenic bacteria, resistant to currently marketed antibacterial agents has reached an alarming level in many countries (Akbas and Berber 2005). There has also been a rapid spread in primary and opportunistic fungal infections because of the increased number of immune-compromised patients (AIDS, cancer and organ transplants). *Candida albicans* is one of the most commonly found opportunistic fungi responsible for such type of infections. (Turan-Zitouni et al. 2005). Infections caused by these microorganisms pose a serious challenge to the medical community and highlight the importance and an urgent need for new, more potent and selective non-traditional antimicrobial remedies.

Among various heterocycles that have been explored for developing pharmaceutically important molecules, isoxazoles, their bioisosteric thiazoles and their fused heterocyclic ring systems have attracted considerable attention. In this view, a wide range of chemotherapeutic actions have been ascribed to isoxazole derivatives such as antibacterial (Padmaja et al. 2009; Varshney et al. 2009; Lamani et al. 2009; Rajanarendara et al. 2010), antifungal (Santos et al. 2010), antimycobacterial (Moraski et al. 2010; Changtam et al. 2010), nematocidal (Srinivas et al. 2010) and antiviral (Deng et al. 2009) activities, in addition to well documented anticancer potentials (Amolins et al. 2009; Kamal et al. 2010; Diana et al. 2010). Similarly, thiazole-

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containing compounds were reported to contribute to a variety of chemotherapeutic potentials including their antiviral activity against hepatitis-C (Yan et al. 2007), AIDS (HIV) viruses (Sharmeen et al. 2001; Rollas and Kucukguzel 2007; Zeng et al. 2008; Vicini et al. 2009), antimicrobial (Pagani et al. 2000; Vicini et al. 2000, 2002; Kang et al. 2003; Zani et al. 2004), antiparasitic (Balliano et al. 2009), as well as their possible antiproliferative (Geronikaki et al. 2004; Clerici et al. 2006; Vicini et al. 2006) activities.

In view of the above-mentioned facts, and in continuation of our interest in the discovery of novel heterocycles endowed with potential chemotherapeutic activities (Rostom 2006, 2010; Rostom et al. 2003, 2009a, b, 2011; Faidallah and Khan 2012; Faidallah et al. 2007, 2011, 2012, 2013; Al-Saadi et al. 2008a, b), we aim to synthesize, some novel isoxazoles, thiazoles and some derived heterocyclic ring systems having potent antimicrobial activities. The proposed compounds were designed to contain some pharmacophores such as urea, thiourea, sulfonylurea(thiourea) and some derived functionalities that are believed to be responsible for the anticipated biological activities (Croitoru et. al. 2004; Limban et al. 2008, 2009), with an aim of discovering new lead structures that might possibly be used in designing new potent and selective antimicrobial agents.

Materials and methods

Chemistry

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu FT-IR 8400S IR spectrophotometer using the KBr pellet technique. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FT NMR spectrometer using tetramethylsilane as the internal standard and DMSO-d_6 as a solvent (Chemical shifts in δ, ppm). Splitting patterns were designated as follows: *s*: singlet; *d*: doublet; *m*: multiplet; *q*: quartet. Mass spectra were measured on a GCM-Q 1000 Ex spectrometer. Elemental analyses were performed on a 2400 Perkin Elmer Series 2 analyzer and the found values were within ±0.4 % of the theoretical values. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254. Ethyl 5-acetyl-3-methylisoxazole-4-carboxylate (**1**) and ethyl 5-acetyl-2-methylthiazole-4-carboxylate (**16**) were purchased from Maybridge Chemical Company Limited, UK (Table 1).

3,7-Dimethyl-5*H*-isoxazolo[4,5-*d*]pyridazin-4-one (**2**)

A solution of ethyl 5-acetyl-3-methylisoxazole-4-carboxylate (**1**) (1.97 g, 10 mmol) in ethanol (25 ml) was refluxed with the hydrazine hydrate (1.0 ml) for 2 h. The product was precipitated after evaporation of some solvent from the reaction mixture. The resulting solid was filtered, washed with cold ethanol, and recrystallized from hot ethanol as needles.

IR (KBr, ν_{max}, cm^{-1}) 1,662 (CO), 3,285 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.49 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 12.90 (s, 1H, NH); MS *m/z* (relative intensity) 165 (M^+, 100), 137 (M–CO, 30),123 (M–NCO, 25), 109 (22), 94 (4), 81 (8), 66 (18) 50(12), 43 (8).

5-(Substituted-carbamoyl)-3,7-dimethyl-4-oxo-4*H*-isoxazolo[4,5-*d*]pyridazines (**3a–c**)

A mixture of the pyridazine derivative **2** (0.98 g, 5 mmol), anhydrous K_2CO_3 (0.01 mol) in dry acetone (50 ml) was stirred. A solution of the appropriate isocyanate (5 mmol) in dry acetone (5.0 ml) was added dropwise with a dropping funnel while stirring the reaction mixture. After addition, the reaction mixture was stirred and refluxed for 16 h. The reaction mixture was cooled to room temperature and acetone was removed under reduced pressure. The solid residue obtained after the evaporation of the solvent was dissolved in H_2O. The crude product was separated upon acidification with dilute HCl, which was filtered and recrystallized from ethanol.

3a: IR (KBr, ν_{max}, cm^{-1}) 1,654 (CO), 1,663 (CO), 3,289 (NH); ^{1}H NMR (DMSO-d_6) δ: 0.99–1.92 (m, 11H, cyclohexyl H), 2.46 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 10.23 (s, 1H, NH).
3b: IR (KBr, ν_{max}, cm^{-1}) 1,656 (CO), 1,666 (CO), 3,275 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.42 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 7.11–7.35 (m, 5H, C_6H_5), 9.88 (s, 1H, NH).
3c: IR (KBr, ν_{max}, cm^{-1}) 1,655 (CO), 1,662 (CO), 3,282 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.39 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 6.98–7.30 (m, 4H, ArH), 9.85 (s, 1H, NH).

5-(Substituted-thiocarbamoyl)-3,7-dimethyl-4-oxo-4*H*-isoxazolo[4,5-*d*]pyridazines (**4a–d**)

A mixture of the pyridazine derivative **2** (0.98 g, 5 mmol), anhydrous K_2CO_3 (0.01 mol) in dry acetone (50 ml) was stirred. A solution of the appropriate isothiocyanate (5 mmol) in dry acetone (5 ml) was added dropwise with a dropping funnel. After stirring and refluxing the mixture for 10 h, acetone was removed under reduced pressure. The resulting solid residue was dissolved in water and acidified

Table 1 Physical and analytical data of the compounds

Compound	R or R′	Yield (%)	m.p. (°C)	Mol. formula	Calc. %			Found %		
					C	H	N	C	H	N
2	H	82	257	$C_7H_7N_3O_2$	50.91	4.27	25.44	50.95	4.30	25.60
3a	Cyclohexyl	82	220	$C_{14}H_{18}N_4O_3$	57.92	6.25	19.30	58.02	6.36	19.42
3b	C_6H_5	85	228	$C_{14}H_{12}N_4O_3$	59.15	4.25	19.71	59.26	4.37	19.90
3c	*p*-ClC_6H_4	88	252	$C_{14}H_{11}ClN_4O_3$	52.76	3.48	17.58	52.86	3.50	17.62
4a	CH_3	74	254	$C_9H_{10}N_4O_2S$	45.37	4.23	23.51	45.46	4.34	23.48
4b	C_6H_5	80	186	$C_{14}H_{12}N_4O_2S$	55.99	4.03	18.65	56.00	4.12	18.70
4c	*p*-ClC_6H_4	78	204	$C_{14}H_{11}ClN_4O_2S$	50.33	3.31	16.73	50.30	3.42	16.84
4d	*p*-FC_6H_4	76	162	$C_{14}H_{11}FN_4O_2S$	52.82	3.48	17.60	52.92	3.53	17.72
5		69	245	$C_9H_6F_3N_3O_3$	41.39	2.32	16.09	41.50	3.12	16.15
6		72	272	$C_9H_6F_3N_5O$	42.03	2.35	27.23	42.12	2.42	27.24
7	*p*-$NH_2SO_2C_6H_4$	88	176	$C_{13}H_{12}N_4O_4S$	48.74	3.77	14.49	48.82	3.88	14.52
8		70	58	$C_9H_{12}N_2O_4$	50.94	5.70	13.20	51.11	5.81	13.34
9		65	212	$C_7H_6N_2O_3$	50.61	3.64	16.86	50.75	3.80	16.78
10a	Cyclohexyl	76	170	$C_{20}H_{23}N_5O_5S$	53.92	5.20	15.72	54.02	5.32	15.67
10b	C_6H_5	82	162	$C_{20}H_{17}N_5O_5S$	54.66	3.90	15.94	54.65	4.11	16.10
10c	*p*-ClC_6H_4	70	255	$C_{20}H_{16}ClN_5O_5S$	50.69	3.40	14.78	50.75	3.56	14.59
11a	CH_3	74	187	$C_{15}H_{15}N_5O_4S_2$	45.79	3.84	17.80	45.88	3.67	17.69
11b	C_6H_5	75	140	$C_{20}H_{17}N_5O_4S_2$	52.74	3.76	15.37	52.65	3.81	15.43
11c	*p*-ClC_6H_4	72	196	$C_{20}H_{16}ClN_5O_4S_2$	49.03	3.29	14.29	48.92	3.43	14.35
11d	*p*-FC_6H_4	66	189	$C_{20}H_{16}FN_5O_4S_2$	50.73	3.41	14.79	50.85	3.34	14.83
11e	COC_6H_5	78	232	$C_{21}H_{17}N_5O_5S_2$	52.16	3.54	14.48	52.21	3.65	14.51
11f	*p*-$CH_3C_6H_4$	64	168	$C_{21}H_{19}N_5O_4S_2$	53.72	4.08	14.92	53.86	4.12	15.00
12a	CH_3	62	160	$C_{23}H_{19}N_5O_4S_2$	55.97	3.88	14.19	55.81	3.72	14.22
12b	C_6H_5	70	188	$C_{28}H_{21}N_5O_4S_2$	60.53	3.81	12.60	60.54	3.72	12.74
12c	*p*-ClC_6H_4	72	176	$C_{28}H_{20}ClN_5O_4S_2$	56.99	3.42	11.87	57.05	3.53	11.97
12d	*p*-FC_6H_4	67	102	$C_{28}H_{20}FN_5O_4S_2$	58.63	3.51	12.21	58.75	3.65	12.34
13a	CH_3	65	160	$C_{17}H_{15}N_5O_5S_2$	47.10	3.49	16.16	47.21	3.52	16.21
13b	C_6H_5	68	175	$C_{22}H_{17}N_5O_5S_2$	53.32	3.46	14.13	53.43	3.54	14.23
13c	*p*-ClC_6H_4	66	154	$C_{22}H_{16}ClN_5O_5S_2$	49.86	3.04	13.21	49.78	3.15	13.22
13d	*p*-FC_6H_4	62	226	$C_{22}H_{16}FN_5O_5S_2$	51.46	3.14	13.64	51.54	3.23	13.54
13e	COC_6H_5	59	207	$C_{23}H_{17}N_5O_6S_2$	52.76	3.27	13.38	52.87	3.36	13.49
14a	C_6H_5	58	159	$C_{22}H_{15}N_5O_6S_2$	51.86	2.97	13.75	51.76	3.04	13.68
14b	*p*-FC_6H_4	60	132	$C_{22}H_{14}FN_5O_6S_2$	50.09	2.68	13.28	50.19	2.77	13.40
15a	C_6H_5	64	178	$C_{22}H_{19}N_5O_4S_2$	54.87	3.98	14.54	54.69	4.11	14.62
15b	*p*-FC_6H_4	61	125	$C_{22}H_{18}FN_5O_4S_2$	52.90	3.63	14.02	53.02	3.76	14.11
15c	*p*-$CH_3C_6H_4$	56	288	$C_{23}H_{21}N_5O_4S_2$	55.74	4.27	14.13	55.87	4.42	14.26
17		78	76	$C_9H_{12}N_2O_3S$	47.37	5.26	12.28	47.45	5.41	12.32
18		68	162	$C_7H_6N_2O_2S$	46.15	3.30	15.38	46.23	3.41	15.42
19		86	254	$C_7H_7N_3OS$	46.41	3.87	23.20	46.34	3.93	23.40
20a	C_6H_5	65	232	$C_{14}H_{12}N_4O_2S$	55.99	4.03	18.65	56.12	4.18	18.75
20b	*p*-ClC_6H_4	68	300	$C_{14}H_{11}ClN_4O_2S$	50.23	3.31	16.74	50.41	3.43	16.87
21a	CH_3	71	220	$C_9H_{10}N_4OS_2$	42.50	3.96	22.03	42.63	4.00	22.21
21b	C_6H_5	62	298	$C_{14}H_{12}N_4OS_2$	53.14	3.82	17.71	53.26	3.92	17.87
21c	*p*-ClC_6H_4	64	148	$C_{14}H_{11}ClN_4OS_2$	47.93	3.16	15.97	48.08	3.22	16.02
22	*p*-$NH_2SO_2C_6H_4$	84	249	$C_{13}H_{12}N_4O_3S_2$	46.43	3.57	16.67	46.36	3.61	16.73
23a	C_6H_5	76	162	$C_{20}H_{17}N_5O_4S_2$	52.74	3.76	15.37	52.82	3.88	15.44
23b	*p*-ClC_6H_4	72	186	$C_{20}H_{16}ClN_5O_4S_2$	49.03	3.29	14.29	49.17	3.40	14.42

Table 1 continued

Compound	R or R′	Yield (%)	m.p. (°C)	Mol. formula	Calc. %			Found %		
					C	H	N	C	H	N
23c	Cyclohexyl	75	206	$C_{20}H_{23}N_5O_4S_2$	52.04	5.02	15.17	52.22	4.98	15.08
24a	CH_3	67	122	$C_{15}H_{15}N_5O_3S_3$	43.99	3.69	17.10	44.12	3.56	17.22
24b	C_6H_5	66	172	$C_{20}H_{17}N_5O_3S_3$	50.94	3.63	14.85	51.10	3.74	15.00
24c	*p*-ClC_6H_4	69	205	$C_{20}H_{16}ClN_5O_3S_3$	47.47	3.19	13.84	47.52	3.05	13.92

with dilute HCl. The precipitated crude product was filtered and recrystallized from ethanol.

4a: IR (KBr, ν_{max}, cm^{-1}) 1,660 (CO), 1,122 (CS), 3,304 (NH); ^1H NMR (DMSO-d_6) δ: 2.28 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 3.35 (s, 3H, NCH_3), 9.22 (s, 1H, NH).
4b: IR (KBr, ν_{max}, cm^{-1}) 1,655 (CO), 1,124 (CS), 3,290 (NH); ^1H NMR (DMSO-d_6) δ: 2.25 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 7.12–7.35 (m, 5H, C_6H_5), 10.22 (s, 1H, NH).
4c: IR (KBr, ν_{max}, cm^{-1}) 1,657 (CO), 1,142 (CS), 3,268 (NH); ^1H NMR (DMSO-d_6) δ: 2.28 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 7.00–7.45 (m, 4H, ArH), 9.88 (s, 1H, NH).
4d: IR (KBr, ν_{max}, cm^{-1}) 1,658 (CO), 1,135 (CS), 3,288 (NH). ^1H NMR (DMSO-d_6) δ: 2.26 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 6.59–6.99 (m, 4H, ArH), 9.28 (s, 1H, NH).

5-Trifluoroacetyl-3,7-dimethyl-4-oxo-4*H*-isoxazolo[4,5-*d*]pyridazines (**5**)

A solution of the pyridazine derivative **2** (1.97 g, 10 mmol), THF (25 ml) was refluxed with trifluoroacetic anhydride (2.1 g, 10 mmol) for 2 h. The reaction mixture was then cooled and poured into water; the precipitated trifluoroacetyl derivative was recrystallized from ethanol as needles. IR (KBr, ν_{max}, cm^{-1}) 1,658 (CO), 1,669 (CO), ^1H NMR (DMSO-d_6) δ: 2.26 (s, 3H, CH_3), 2.46 (s, 3H, CH_3),

3-Trifluoroacetyl-6,9-dimethylisoxazolo[4,5-*d*]-1,2,4-triazolo[4,3-*b*]pyridazine (**6**)

A solution of the appropriate trifluoroacetylpyridazine **5** (2.6 g, 10 mmol) in DMF (20 ml) was refluxed with hydrazine hydrate (0.6 g, 12 mmol) for 4 h. The reaction mixture was then cooled and poured onto ice water. The solid that separated was filtered off, dried, and recrystallized from absolute alcohol as needles. ^1H NMR (DMSO-d_6) δ: 2.36 (s, 3H, CH_3), 2.42 (s, 3H, CH_3),

3,7-Dimethyl-5-(4-sulfonamidophenyl)-5*H*-isoxazolo[4,5-*d*]pyridazine-4-one (**7**)

A solution of ethyl 5-acetyl-3-methylisoxazole-4-carboxylate (**1**) (1.97 g, 10 mmol) in ethanol (25 ml) was refluxed with the *p*-sulphamylphenyl hydrazine (2.2 g, mmol) for 4 h. The pyridazine which separated after concentration of the reaction mixture was filtered, washed with cold ethanol, and recrystallized from ethanol as needles.

IR (KBr, ν_{max}, cm^{-1}) 1,665 (CO), 3,337 and 3,200 (NH_2); ^1H NMR (DMSO-d_6) δ: 2.35 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 7.39–8.20 (m, 6H, Ar + NH_2). MS *m/z* (relative intensity) 320 (M^+, 25), 292 (M–CO, 28), 240 (M–SO_2NH_2, 14), 184 ($N_2C_6H_4SO_2NH_2$, 21), 170 ($^+NC_6H_4SO_2NH_2$, 16), 164 (M–$C_6H_4SO_2NH_2$, 10), 156 ($C_6H_4SO_2NH_2$, 40), 142 (40), 123 (100), 108 (9), 107 (14), 92 (44), 81(5), 85 (11), 80 (12), 75 (37), 66 (15), 50 (29).

Ethyl 5-acetyl-3-methylisoxazole-4-carboxylate oxime (**8**)

A solution of **1** (0.39 g, 0.002 mol) in ethanol (20 ml) was refluxed with hydroxylamine hydrochloride (0.15 g, 0.0022 mol) and sodium acetate (0.18 g, 0.0022 mol) for 3 h. The oxime which separated after dilution with water was recrystallized from diluted ethanol as needles; IR (KBr, ν_{max}, cm^{-1}) 1,735 (CO), 3,310 (OH); ^1H NMR ($CDCl_3$) δ: 1.38 (t, $J = 6$ Hz, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 4.32 (q, $J = 6$ Hz, 2H, CH_2).

3,7-Dimethyl-4*H*-isoxazolo[5,4-*d*][1,2]oxazin-4-one (**9**)

A solution of **1** (0.39 g, 0.002 mol) in pyridine (10 ml) was refluxed with hydroxylamine hydrochloride (0.15 g, 0.0022 mol) for 6 h. The oxazinone **4** separated on dilution with water and was recrystallized from ethanol as needles. IR (KBr, ν_{max}, cm^{-1}) 1,750 (CO); ^1H NMR ($CDCl_3$) δ: 2.48 (s, 3H, CH_3), 2.68 (s, 3H, CH_3); MS *m/z* (relative intensity) 166 (M^+, 65), 125 (M–CH_3CN, 41), 109 (10), 97 (5), 98 (25), 81 (10), 66 (18), 44 (22), 41 (100).

p-(3,7-Dimethyl-4-oxo-4*H*-isoxazolo[4,5-*d*]pyridazine-5-yl)benzenesulfonylureas (**10**)

A mixture of **7** (3.2 g, 10 mmol) and anhydrous K_2CO_3 (20 mmol) in dry acetone (25 ml) was stirred and treated with the appropriate isocyanate (11 mmol). After the mixture was

stirred and refluxed for 10 h, acetone was removed under reduced pressure, and the solid mass dissolved in water and acidified with 2 N HCl. The crude product was purified by recrystallization from ethanol as needles.

10a: IR (KBr, ν_{max}, cm^{-1}) 1,658 (CO), 1,666 (CO), 3,295 (NH); ^{1}H NMR (DMSO-d_6) δ: 1.11, 1.49, 3.54 (3 m, 11H, cyclohexyl H), δ 2.38 (s, 3H, CH_3), 2.86 (s, 3H, CH_3), 7.82–8.04 (m, 4H, Ar–H), 8.23 (s, 1H, NH), 8.98 (s, 1H, NH).
10b: IR (KBr, ν_{max}, cm^{-1}) 1,658 (CO), 1,663 (CO), 3,295 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.28 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 7.19–8.15 (m, 5H, C_6H_5), 8.98 (s, 1H, NH), 9.62 (s, 1H, NH).
10c: IR (KBr, ν_{max}, cm^{-1}) 1,659 (CO), 1,665 (CO), 3,282 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 7.47–7.99 (m, 4H, Ar–H), 8.68 (s, 1H, NH) 0.9.45 (s, 1H, NH).

p-(3,7-Dimethyl-4-oxo-4*H*-isoxazolo [4,5-*d*]pyridazine-5-yl)benzenesulfonylthioureas (**11**)

A mixture of pyridazine derivative **7** (3.2 g, 10 mmol) and anhydrous K_2CO_3 (3.2 g, 10 mmol) in dry acetone (25 ml) was stirred and treated with the appropriate isothiocyanate (12 mmol). After the mixture was stirred and refluxed for 10 h, acetone was removed under reduced pressure, and the solid mass dissolved in water and acidified with 2 N HCl. The crude product was purified by recrystallization from ethanol as needles.

11a: IR (KBr, ν_{max}, cm^{-1}) 1,662 (CO), 1,124 (CS), 3,308 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.29 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 3.33 (s, 3H, NCH_3), 7.84–8.00 (m, 4H, Ar–H), 8.54 (s, 1H, NH), 9.22 (s, 1H, NH).
11b: IR (KBr, ν_{max}, cm^{-1}) 1,658 (CO), 1,120 (CS), 3,322 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.42 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 6.81–7.89 (m, 9H, C_6H_5), 8.66 (s, 1H, NH), 10.22 (s, 1H, NH).
11c: IR (KBr, ν_{max}, cm^{-1}) 1,660 (CO), 1,132 (CS), 3,328 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.37 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 6.76–8.01 (m, 8H, ArH), 8.78 (s, 1H, NH), 9.58 (s, 1H, NH).
11d: IR (KBr, ν_{max}, cm^{-1}) 1,656 (CO), 1,134 (CS), 3,320 (NH). ^{1}H NMR (DMSO-d_6) δ: 2.35 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 6.76–8.11 (m, 8H, ArH), 8.55 (s, 1H, NH), 9.28 (s, 1H, NH).
11e: IR (KBr, ν_{max}, cm^{-1}) 1,660 (CO), 1,128 (CS), 3,325 (NH). ^{1}H NMR (DMSO-d_6) δ: 2.27 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 6.68–7.88 (m, 9H, ArH), 8.55 (s, 1H, NH), 9.28 (s, 1H, NH).
11f: IR (KBr, ν_{max}, cm^{-1}) 1,660 (CO), 1,138 (CS), 3,320 (NH). ^{1}H NMR (DMSO-d_6) δ: 2.28 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 6.48–7.87 (m, 8H, ArH), 8.55 (s, 1H, NH), 9.28 (s, 1H, NH).

3-Substituted-2-[*p*-(3,7-dimethyl-4-oxo-4*H*-isoxazolo[4,5-*d*]pyridazine-5-yl)benzene-sulfonylimino]-1,3-thiazolines (**12a–d**)

A solution of the corresponding thiourea derivative **11** (10 mmol) in absolute ethanol (25 ml) was refluxed with the α-bromoacetophenone (1.99 g, 10 mmol) and sodium acetate (20 mmol) for 2 h. The reaction mixture was then cooled and poured into water; the precipitated thiazoline was recrystallized from ethanol as needles.

12a: IR (KBr, ν_{max}, cm^{-1}) 1,659 (CO), 3,312 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.29 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 3.41 (s, 3H, NCH_3), 7.40–7.89 (m, 9H, Ar–H).
12b: IR (KBr, ν_{max}, cm^{-1}) 1,656 (CO), 3,320 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.35 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 6.85–7.99 (m, 14H, C_6H_5).
12c: IR (KBr, ν_{max}, cm^{-1}) 1,661 (CO), 3,324 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.31 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 6.49–7.78 (m, 12H, ArH).
12d: IR (KBr, ν_{max}, cm^{-1}) 1,659 (CO), 3,318 (NH). ^{1}H NMR (DMSO-d_6) δ: 2.29 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 7.37–8.05 (m, 12H, ArH).

3-Substituted-2-[*p*-(3,7-dimethyl-4-oxo-4*H*-isoxazolo[4,5-*d*]pyridazine-5-yl)benzene-sufonylimino]-4-oxothiazolidines (**13a–e**)

A mixture of **11** (10 mmol), ethyl bromoacetate (1.67 g, 10 mmol) and sodium acetate (20 mmol) in absolute ethanol (30 ml) was refluxed for 2 h. The reaction mixture was then filtered while hot, concentrated and allowed to cool. The product obtained was recrystallized from ethanol as needles.

13a: IR (KBr, ν_{max}, cm^{-1}) 1,657 (CO), 1,710 (CO), 3,321 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.27 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 3.32 (s, 3H, NCH_3), 7.43–7.86 (m, 4H, Ar–H).
13b: IR (KBr, ν_{max}, cm^{-1}) 1,658 (CO), 1,714 (CO), 3,326 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.29 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 6.75–7.94 (m, 9H, C_6H_5).
13c: IR (KBr, ν_{max}, cm^{-1}) 1,663 (CO), 1,708 (CO), 3,328 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.32 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 6.47–7.87 (m, 8H, ArH).
13d: IR (KBr, ν_{max}, cm^{-1}) 1,660 (CO), 1,711 (CO), 3,328 (NH). ^{1}H NMR (DMSO-d_6) δ: 2.31 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 7.42–8.09 (m, 8H, ArH).
13e: IR (KBr, ν_{max}, cm^{-1}) 1,664 (CO), 1,675 (CO), 1,711 (CO), 3,323 (NH). ^{1}H NMR (DMSO-d_6) δ: 2.29 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 6.65–7.88 (m, 9H, ArH).

3-Substituted-2-[*p*-(3,7-dimethyl-4-oxo-4*H*-isoxazolo[4,5-*d*]pyridazine-5-yl)benzene-sulfonylimino]-4,5-dioxothiazolidines (**14a**, **b**)

A mixture of the appropriate thiourea **11** (10 mmol), diethyl oxalate (1.46 g, 10 mmol) and sodium acetate (20 mmol) in absolute ethanol (25 ml) was refluxed for 2 h. The thiazine which separated on cooling was recrystallized from ethanol as needles.

14a: IR (KBr, ν_{max}, cm^{-1}) 1,661 (CO), 1,675 (CO), 1,712 (CO); ^1H NMR (DMSO-d_6) δ: 2.29 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 6.73–7.96 (m, 9H, C_6H_5).
14b: IR (KBr, ν_{max}, cm^{-1}) 1,663 (CO), 1,678 (CO), 1,715 (CO); ^1H NMR (DMSO-d_6) δ: 2.31 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 6.45–7.68 (m, 8H, ArH)).

3-Substituted-2-[*p*-(3,7-dimethyl-4-oxo-4*H*-isoxazolo[4,5-*d*]pyridazine-1-yl)benzene-sulfonylimino]thiazolidines (**15a**–**c**)

A solution of **11** (10 mmol) in absolute ethanol (20 ml) was refluxed with 1,2-diiodoethane (2.8 g, 10 mmol) and sodium acetate (20 mmol) for 2 h. The reaction mixture was then cooled and poured into water; the precipitated thiazine was recrystallized from ethanol as needles.

15a: IR (KBr, ν_{max}, cm^{-1}) 1,661 (CO); ^1H NMR (DMSO-d_6) δ: 2.27 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 3.52 (t, 2H, CH_2), 3.73 (t, 2H, CH_2), 6.63–7.94 (m, 9H, C_6H_5).
15b: IR (KBr, ν_{max}, cm^{-1}) 1,663 (CO); ^1H NMR (DMSO-d_6) δ: 2.28 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 3.54 (t, 2H, CH_2), 3.75 (t, 2H, CH_2), 6.47–7.78 (m, 8H, Ar).

Ethyl-5-acetyl-2-methylthiazole-4-carboxylate oxime (**17**)

A solution of **16** (0.39 g, 0.002 mol) in ethanol (20 ml) was refluxed with hydroxylamine hydrochloride (0.69 g, 10 mmol) and sodium acetate (0.82 g, 10 mmol) for 3 h. The oxime which separated after dilution with water was recrystallized from diluted ethanol as needles; IR (KBr, ν_{max}, cm^{-1}) 1,740 (CO), 3,277 (OH); ^1H NMR ($CDCl_3$) δ: 1.40 (t, $J = 6$ Hz, 3H, CH_3), 2.55 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 4.45. (q, $J = 6$ Hz, 2H, CH_2).

2,7-Dimethyl-4*H*-thiazolo[5,4-*d*][1,2]oxazin-4-one (**18**)

A solution of **16** (2.1 g, 10 mmol) in pyridine (10 ml) was refluxed with hydroxylamine hydrochloride (0.69 g, 10 mmol) for 6 h. The oxazinone **18** separated on dilution with water and was recrystallized from ethanol as needles. IR (KBr, ν_{max}, cm^{-1}) 1,745 (CO); ^1H NMR ($CDCl_3$) δ: 2.42 (s, 3H, CH_3), 2.70 (s, 3H, CH_3); MS *m/z* (relative intensity) 182 (M^+, 50), 141 (M–CH_3CN, 38), 125 (8), 113 (12), 112 (23), 100 (13), 97 (4), 87(10), 82 (10), 70 (24), 59(100), 57(24).

2,7-Dimethyl-5*H*-thiazolo[4,5-*d*]pyridazin-4-one (**19**)

A solution of ethyl 5-acetyl-2-methylthiazole-4-carboxylate **16** (2.1 g, 10 mmol) in ethanol (25 ml) was refluxed with the hydrazine hydrate (1 ml) for 2 h. The pyridazine which separated after concentration of the reaction mixture was filtered, washed with cold ethanol, and recrystallized from ethanol as needles.

IR (KBr, ν_{max}, cm^{-1}) 1,655 (CO), 3,293 (NH); ^1H NMR (DMSO-d_6) δ: 2.50 (s, 3H, CH_3), 2.88 (s, 3H, CH_3), 12.82 (s, 1H, NH); MS *m/z* (relative intensity) 181 (M^+, 100), 153 (M–CO, 28), 125 (M–NCO, 17), 110 (12), 97 (4), 84 (16), 66 (22), 50 (12), 43 (8).

5-(Substituted-carbamoyl)-2,7-dimethyl-4-oxo-4*H*-thiazolo[4,5-*d*]pyridazines (**20a**, **b**)

A mixture of the thiazolopyridazine derivative **19** (1.8 g, 5 mmol), anhydrous K_2CO_3 (0.01 mol) in dry acetone (50 ml) was stirred. A solution of the appropriate isocyanate (5 mmol) in dry acetone (5 ml) was added dropwise with a dropping funnel while stirring the reaction mixture. After addition, the reaction mixture was stirred and refluxed for 16 h. The reaction mixture was cooled to room temperature and acetone was removed under reduced pressure. The solid residue obtained after the evaporation of the solvent was dissolved in H_2O. The crude product was separated upon acidification with dilute HCl, which was filtered and recrystallized from ethanol.

20a: IR (KBr, ν_{max}, cm^{-1}) 1,652 (CO), 1,664 (CO), 3,268 (NH); ^1H NMR (DMSO-d_6) δ: 2.28 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 7.19–7.61 (m, 5H, ArH), 9.95 (s, 1H, NH).
20b: IR (KBr, ν_{max}, cm^{-1}) 1,659 (CO), 1,667 (CO), 3,279 (NH); ^1H NMR (DMSO-d_6) δ: 2.29 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 7.46–7.75 (m, 4H, C_6H_5), 9.78 (s, 1H, NH).

5-(Substituted-thiocarbamoyl)-2,7-dimethyl-4-oxo-4*H*-thiazolo[4,5-*d*]pyridazines (**21a**–**c**)

A mixture of the thiazolopyridazine derivative **19** (1.8 g, 5 mmol), anhydrous K_2CO_3 (0.01 mol) in dry acetone (50 ml) was stirred. A solution of the appropriate isothiocyanate (5 mmol) in dry acetone (5 ml) was added dropwise with a dropping funnel. After stirring and refluxing the mixture for 10 h, acetone was removed under reduced pressure. The resulting solid residue was dissolved in water

Table 2 ^{13}C NMR spectral data[a] of the synthesized compounds

Compound	Ar C	CH_3 and others	CO/CS
2	102.1, 150.5, 156.7, 158.6	8.6, 17.6	169.7
3a	102.3, 152.3, 155.9, 159.5	9.1, 16.9, 23.2, 24.1, 32.4, 46.3 (cyclohexyl C)	160.8, 170.2
3b	100.8, 120.2, 124.7, 127.7, 138.2, 150.6, 156.0, 158.7	9.8, 17. 6	162.3, 172.1
4b	103.7, 124.3, 125.8, 128.0, 139.0, 151.2, 155.4, 158.4	9.5, 18.2	161.8, 189.9
4c	104.3, 123.9, 125.1, 128.9, 138.4, 150.1, 156.3, 159.3	9.4, 18.1	164.8, 192.3
5	100.7, 150.4, 152.4, 158.4	8.5, 17.5	167.8, 170.2
6	100.3, 147.3, 150.1, 158.9, 160.0, 160.8,	11.2, 14.1, 116.2[b]	
7	101.8, 120.5, 125.4, 134.2, 141.9,150.2, 158.7, 160.2	8.9.7, 17.7	166.4
9	100.8, 150.5, 157.7, 164.0	7.4, 21.8	172.2
10a	100.5, 120.4, 125.2, 134.7, 141.3, 150.6, 155.7, 158.4	8.1, 17.6, 21.6, 27.4, 32.62, 46.34 (cyclohexyl C)	163.5, 166.3
10b	100.5, 120.4, 120.5, 124.1, 125.2, 128.7, 138.2, 134.7, 141.3, 150.6, 155.7, 158.4	8.3, 17.7	161.4, 165.9
11b	101.5, 120.2, 120.4, 125.5, 126.2, 128.4, 138.5, 135.2, 141.8, 150.2, 156.3, 158.8	8.7, 18.2	167.7, 179.8
11c	100.9, 120.4, 125.5, 126.5, 129.2, 129.9, 134.3, 137.7, 141.7, 150.5, 156.3, 158.8	8.6, 18.3	166.9, 180.1
11f	100.8, 120.3, 121.4, 125.8, 126.4, 129.5, 133.5, 136.4, 142.5, 150.3, 156.8, 159.2	8.4, 17.8, 20.8	167.1, 180.5
12b	98.9, 100.8, 115.1, 118.6, 120.3, 121.4, 125.2, 125.8, 126.1, 126.4, 127.4, 128.4, 135.5, 142.5, 152.4, 150.3, 156.8, 158.9, 162.4	8.6, 17.9	166.9
13b	100.1, 120.3, 121.4, 124.2, 126.6, 128.7, 134.1, 140.6, 143.4, 150.5, 156.7, 158.6, 163.3	7.9, 17.6, 32.8[c]	165.6, 168.6
13c	101.2, 120.5, 125.2, 126.1, 128.8, 129.7, 133.8, 137.3, 141.4, 150.2, 156.8, 158.9, 163.5	8.2, 17.7, 32.8[c]	166.2, 167.9
14a	100.5, 120.1, 121.8, 124.3, 126.4, 128.4, 134.3, 140.3, 143.5, 150.5, 157.0, 158.6, 164.2	7.9, 17.6	161.4, 168.2, 186.6
15a	100.1, 120.7, 121.6, 124.5, 126.6, 128.7, 134.2, 140.1, 143.7, 150.4, 157.2, 158.9, 163.4	7.7, 17.7, 30.8[c], 56.8[c]	166.9
15c	100.2, 120.7, 121.9, 125.6, 126.4, 129.3, 134.2, 136.7, 141.8, 150.5, 156.9, 158.6, 163.9	8.1, 17.8, 20.6, 30.5[c], 55.9[c]	167.2
17	119.3, 143.5, 164.6, 166.3	13.6, 15.4, 15.7, 59.1[c]	167.6
18	120.1, 142.9, 164.2, 165.6	15.8, 21.4	172.6
19	119.5, 141.5, 155.2, 166.3	15.2, 17.4	170.2
20a	119.3, 120.2, 124.3, 128.6, 138.5, 143.5, 155.6, 166.0	15.5, 17.2	156.4, 167.8
21b	119.3, 124.2, 125.5, 128.8, 139.5, 143.5, 155.3, 166.2	15.4, 18.2	169.5, 184.4
22	119.8, 120.7, 125.9, 134.8, 141.5, 143.3, 154.9, 166.1	15.6, 18.0	165.7
23a	119.5, 120.4, 120.6, 124.2, 125.9, 128.8, 134.8, 138.4, 141.5, 143.0, 156.0, 166.3	15.4, 17.7	157.8, 166.2
24a	119.2, 120.7, 125.4, 134.9, 141.4, 143.6, 164.3, 166.2	15.4, 17.7, 33.7[d]	166.3, 186.6
24b	119.4, 120.6, 124.4, 125.3, 125.7, 128.9, 134.8, 139.3, 141.5, 143.3, 154.9, 166.1	15.3, 17.8	165.8, 186.2
24c	119.6, 120.5, 125.8, 126.7, 129.3, 129.8, 134.6, 137.4, 141.3, 143.6, 154.5, 165.9	15.1, 17.6	166.0, 186.5

[a] Solution in DMSO-d_6/$CDCl_3$, δ in ppm

[b] CF_3

[c] CH_2

[d] N–CH_3

Table 3 MIC (μg/ml) of the active newly synthesized compounds

Compound No.	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
2	100	100	–	–	–
3c	50	100	100	–	–
4b	25	50	100	–	–
4c	12.5	50	50	100	50
4d	12.5	25	25	100	50
5	50	50	100	–	–
6	100	–	–	–	–
7	25	50	50	100	50
10c	12.5	25	50	50	100
11c	12.5	50	25	100	25
11d	6.25	25	12.5	50	12.5
11e	50	100	50	–	–
12d	25	100	50	–	–
13c	25	50	25	50	50
13d	12.5	12.5	12.5	50	25
14b	50	50	50	–	–
15b	50	100	100	–	–
19	50	100	–	–	–
20b	25	50	50	–	–
21b	25	50	25	–	100
21c	25	25	12.5	–	50
22	25	50	50	–	100
23b	50	50	50	–	50
24b	25	50	50	100	25
24c	12.5	12.5	25	100	12.5
A	6.25	12.5	6.25	12.5	–
C	–	–	–	–	6.25

A Ampicillin trihydrate, ***C*** Clotrimazole, – totally inactive (MIC ≥ 200 μg/ml)

and acidified with dilute HCl. The precipitated crude product was filtered and recrystallized from ethanol.

21a: IR (KBr, ν_{max}, cm^{-1}) 1,662 (CO), 1,117 (CS), 3,314 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.26 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 3.32 (s, 3H, NCH_3), 9.28 (s, 1H, NH).
21b: IR (KBr, ν_{max}, cm^{-1}) 1,658 (CO), 1,139 (CS), 3,323 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.24 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 7.18–7.45 (m, 5H, C_6H_5), 10.02 (s, 1H, NH).
21c: IR (KBr, ν_{max}, cm^{-1}) 1,660 (CO), 1,147 (CS), 3,268 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.25 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 7.40–7.72 (m, 4H, ArH), 9.93 (s, 1H, NH).

2,7-Dimethyl-5-(4-sulfonamidophenyl)-4-oxo-4*H*-thiazolo[4,5-*d*]pyridazine (**22**)

A solution of ethyl 5-acetyl-2-methylthiazole-4-carboxylate **16** (2.1 g, 10 mmol) in ethanol (25 ml) was refluxed with the *p*-sulphamylphenyl hydrazine (2.2 g, mmol) for 4 h. The pyridazine which separated after concentration of the reaction mixture was filtered, washed with cold ethanol, and recrystallized from ethanol as needles.

IR (KBr, ν_{max}, cm^{-1}) 1,653 (CO), 3,181 and 3,314 (NH_2); ^{1}H NMR (DMSO-d_6) δ: 2.65 (s, 3H, CH_3), 2.74 (s, 3H, CH_3), 7.35–8.10 (m, 6H, Ar + NH_2). MS *m/z* (relative intensity) 336 (M^+, 25), 308 (M–CO, 32), 256 (M–SO_2NH_2, 12), 228 (18), 184 ($N_2C_6H_4SO_2NH_2$, 18), 170 ($^+NC_6H_4SO_2NH_2$, 18), 164 (M–$C_6H_4SO_2NH_2$, 12), 156 ($C_6H_4SO_2NH_2$, 37), 141 (39), 139 (100), 124 (7), 113 (10), 111 (42), 97 (4), 58 (10), 85 (14), 80 (18), 75 (33), 69 (19), 50 (27).

p-(2,7-Dimethyl-4-oxo-4*H*-thiazolo[4,5-*d*]pyridazin-5-yl)benzenesulfonylureas (**23a–c**)

A mixture of **22** (3.3 g, 10 mmol) and anhydrous K_2CO_3 (20 mmol) in dry acetone (25 ml) was stirred and treated with the appropriate isocyanate (11 mmol). After the mixture was stirred and refluxed for 10 h, acetone was removed under reduced pressure, and the resulting solid mass dissolved in water and acidified with 2 N HCl. The crude product thus obtained was purified by recrystallization from ethanol as needles.

23a: IR (KBr, ν_{max}, cm^{-1}) 1,655 (CO), 1,664 (CO), 3,310 (NH); ^{1}H NMR (DMSO-d_6) δ: 1.12, 1.44, 3.50 (3 m, 11H, cyclohexyl H), δ 2.28 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 7.74–7.92 (m, 4H, Ar–H), 8.25 (s, 1H, NH), 9.21(s, 1H, NH).
23b: IR (KBr, ν_{max}, cm^{-1}) 1,657 (CO), 1,668 (CO), 3,295 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.27 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 7.15–8.01 (m, 9H, C_6H_5), 8.78(s, 1H, NH), 9.02 (s, 1H, NH).
23c: IR (KBr, ν_{max}, cm^{-1}) 1,661 (CO), 1,666 (CO), 3,282 (NH); ^{1}H NMR (DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 7.42–7.95 (m, 8H, Ar–H), 8.58 (s, 1H, NH), 9.65 (s, 1H, NH).

p-(2,7-Dimethyl-4-oxo-4*H*-thiazolo[4,5-*d*]pyridazin-5-yl)benzenesulfonylthioureas (**24a–c**)

A mixture of pyridazine derivative **22** (3.3 g, 10 mmol) and anhydrous K_2CO_3 (3.2 g, 10 mmol) in dry acetone (25 ml) was stirred and treated with the appropriate isothiocyanate (12 mmol). The mixture was stirred and refluxed for 10 h. After cooling the reaction mixture to room temperature the solvent acetone was removed in vacuo. The resulting precipitate was dissolved in water and neutralized with 2 N HCl. The crude solid product thus obtained was purified by recrystallization from ethanol as needles.

24a: IR (KBr, ν_{max}, cm^{-1}) 1,658 (CO), 1,124 (CS), 3,320 (NH); ^1H NMR (DMSO-d_6) δ: 2.26 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 2.47 (s, 3H, N–CH_3), 6.85–8.00 (m, 9H, C_6H_5), 8.64 (s, 1H, NH), 9.36 (s, 1H, NH).
24b: IR (KBr, ν_{max}, cm^{-1}) 1,656 (CO), 1,125 (CS), 3,322 (NH); ^1H NMR (DMSO-d_6) δ: 2.28 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 6.87–7.99 (m, 9H, C_6H_5), 8.62 (s, 1H, NH), 9.34 (s, 1H, NH).
24c: IR (KBr, ν_{max}, cm^{-1}) 1,657 (CO), 1,130 (CS), 3,335 (NH); ^1H NMR (DMSO-d_6) δ: 2.26 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 6.74–8.00 (m, 8H, ArH), 8.58 (s, 1H, NH), 9.28 (s, 1H, NH).

Biological evaluation

In vitro antibacterial and antifungal activities

Inhibition-zone (IZ) measurements

All the newly synthesized compounds **2**–**24** were evaluated for their in vitro antimicrobial activity against *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6051) as examples of Gram positive bacteria, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) as examples of Gram negative bacteria, *C. albicans* (ATCC 10231) and *Aspergillus niger* (recultured) as representatives of fungi. Agar-diffusion method was used for the determination of the preliminary antibacterial and antifungal activity. Ampicillin trihydrate and Clotrimazole were used as reference drugs. The results were recorded for each tested compound as the average diameter of IZ of bacterial or fungal growth around the discs in mm. The minimum inhibitory concentration (MIC) measurement was determined for compounds that showed significant growth IZ ($\geq$14 mm) using the two-fold serial dilution method (Scott 1989). The MIC (μg/ml) values of the active compounds against the tested microbial strains are recorded in Table 3.

MIC measurement

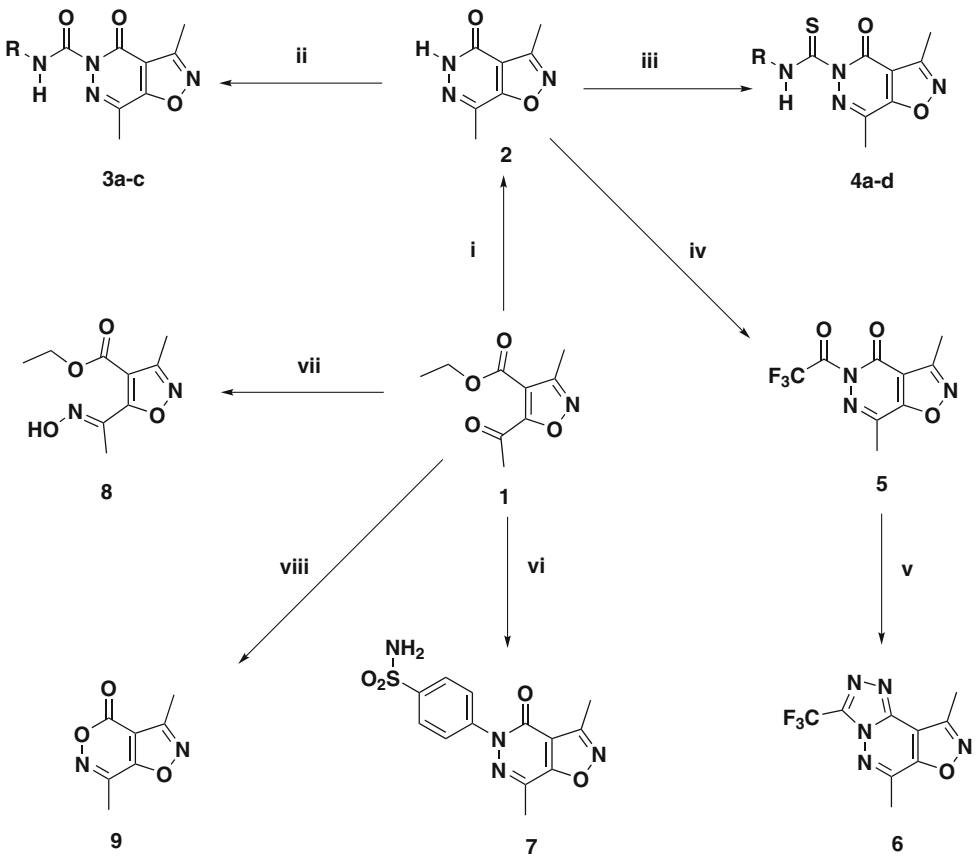
The MIC of the most active compounds were measured using the twofold serial broth dilution method. The test organisms were grown in their suitable broth: 24 h for bacteria and 48 h for fungi at 37 °C. Twofold serial dilutions of solutions of the test compounds were prepared using 200, 100, 50, 25, and 12.5 μg/ml. The tubes were then inoculated with the test organisms; each 5 ml received 0.1 ml of the above inoculums and were incubated at 37 °C for 48 h. Then, the tubes were observed for the presence or absence of microbial growth. The MIC values of the prepared compounds are listed in Table 3.

Results and discussion

Chemistry

The synthetic strategies adopted for the preparation of the intermediate and target compounds are described in Schemes 1, 2, and 3. In Scheme 1, reaction of **1** with hydrazine derivatives afforded the 3,7-dimethylisoxazolo[5,4-d]pyridazin-4(5H)-one (**2**) and 3,7-dimethyl-5-(4-sulfonamidophenyl)-5H-isoxazolo[4,5-*d*]pyridazine-4-one (**7**). The IR spectra of these pyridazines showed carbonyl absorption at 1,662–1,665 cm^{-1}. Their structure was further confirmed from their ^1H NMR which showed two methyl singlets each of three proton intensity at δ 2.35–2.49 and 2.44–2.58 ppm. Condensation of the pyridazine derivative **2** with isocyanates and isothiocyanates afforded the corresponding carbamoyl (**3a**–**c**) and thiocarbamoyl (**4a**–**d**) derivatives respectively. The IR spectra of the carbamoyl derivatives (**3**) exhibited distinguishable urea carbonyl absorptions at 1,663–1,666 cm^{-1} whereas **4** revealed a distinct thiocarbonyl band at 1,122–1,142 cm^{-1} as well as the pyridazinone carbonyl band at 1,654–1,660 cm^{-1}. Alternatively, reaction of the pyridazine derivative **2** with trifluoroacetic anhydride in THF afforded the corresponding trifluoroacetyl derivative **5**, which could be successfully cyclized to 3-trifluoroacetyl-6,9-dimethylisoxazolo[4,5-*d*]-1,2,4-triazolo[4,3-b]pyridazine (**6**) when treated with hydrazine hydrate. The IR spectra of the trifluoroacetyl derivative **5** exhibited two carbonyl absorptions at 1,658 and 1,669 cm^{-1}, which were missing in the IR spectrum of the tricyclic derivative **6**. Additionally, reaction of **1** with hydroxylamine hydrochloride gave ethyl 5-acetyl-3-methylisoxazole-4-carboxylate oxime (**8**) or 3,6-dimethyl-7-oxoisoxazolo[4,5-*d*]-1,2-oxazine (**9**) depending on the reaction conditions (see "Materials and methods" section). Consistent with the assigned structure, the ^1H NMR spectrum of oxime **8** exhibited besides two methyl groups at δ 2.45–2.48 and 2.56–2.68, a triplet and a quartet at δ 1.38 and 4.32 for the CH_3 and CH_2 respectively, of the ester group, which were missing in the oxazine derivative **9**. Condensation of the 4-sulfonamidophenylpyridazine derivative **7** with the appropriate isocyanate and isothiocyanate in dry acetone yielded the corresponding benzenesulfonylurea (**10**) and thiourea (**11**) derivatives respectively (Scheme 2). Their IR spectra exhibited two bands at 1,330–1,362 cm^{-1} and 1,148–1,158 cm^{-1} due to SO_2N group in addition to the urea carbonyl band at 1,663–1,666 cm^{-1} in case of **10** and a thiourea carbonyl absorption at 1,120–1,138 cm^{-1}in the case of **11**. It has been reported that condensation of *N*,*N*′-disubstituted thiourea with chloroacetic acid, its chloride or α-bromo esters afforded 2-imino-4-oxothiazolidines, and the reaction proceeds through the intermediate formation of the cyclic pseudothiohydantoic acid. Therefore, cyclization of

Scheme 1 Synthesis of compounds **2–9**



3: R = **a:** cyclo-C_6H_{11}, **b:** C_6H_5, **c:** 4-Cl-C_6H_4
4: R = **a:** CH_3, **b:** C_6H_5, **c:** 4-Cl-C_6H_4, **d:** 4-F-C_6H_4

Reagents and reaction conditions:
i: $NH_2NH_2.H_2O$; **ii:** RNCO, reflux; **iii:** RNCS, reflux; **iv:** trifluoroacetic anhydride, reflux; **v:** $NH_2NH_2.H_2O$; **vi:** 4-sulfamylphenylhydrazine.HCl, reflux; **vii:** $NH_2OH.HCl$, reflux; **viii:** $NH_2OH.HCl$, pyridine, reflux

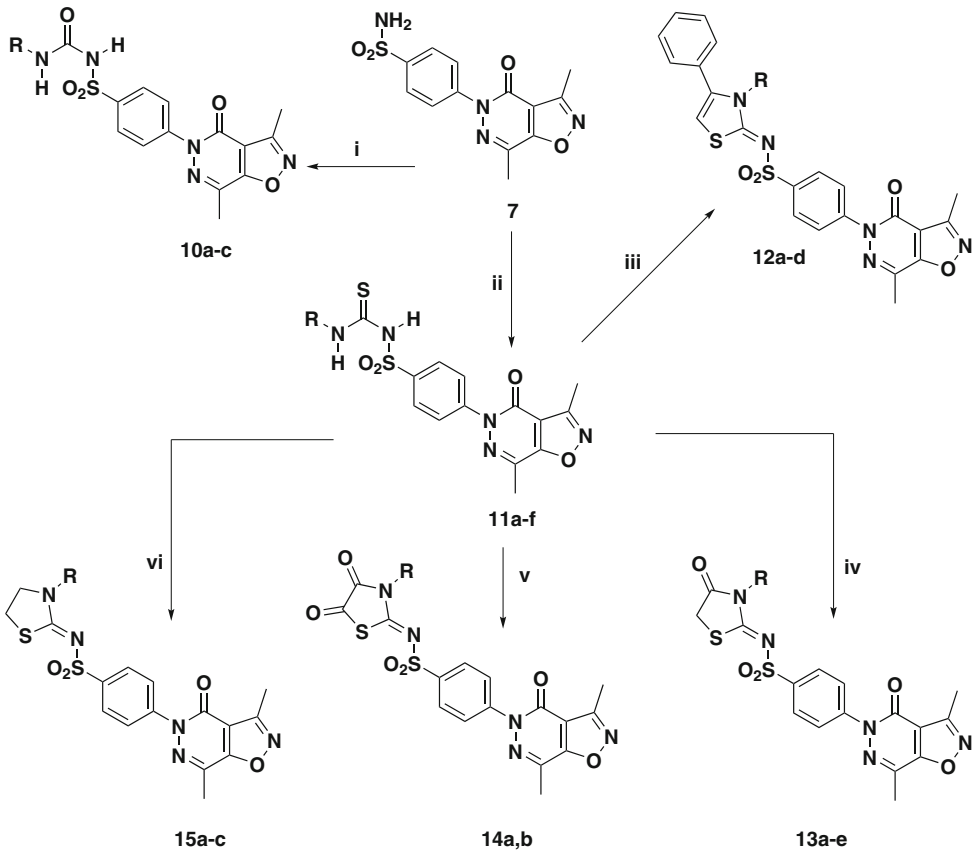
the thiourea derivatives (**11**) with α-bromoacetophenone, ethyl bromoacetate, diethyl oxalate and 1,2-diiodoethane, yielded the corresponding thiazoline (**12**), 4-oxothiazolidine (**13**), 4,5-*di*-oxothiazolidine (**14**) and thiazolidine (**15**) respectively. IR spectra of **13** and **14** showed cyclic carbonyl absorptions at 1,710–1,715 cm^{-1} and two other absorption bands at 1,335–1,344 cm^{-1} and 1,150–1,164 cm^{-1} for the SO_2N group. The structures of **11**–**15** were further supported by their ^1H NMR ("Materials and methods" section) and ^{13}C NMR spectral data (Table 2). With regard to the structurally related thiazole congeners, reaction of the readily available ethyl 5-acetyl-5-methylthiazole-4-carboxylate (**16**) with hydrazine derivatives gave 2,7-dimethylthiazolo[5,4-*d*]pyridazin-4(5*H*)-one (**19**) and 5-(4-sulfonamidophenyl)-2,7-dimethylthiazolo[5,4-*d*]pyridazin-4(5*H*)-one (**22**) (Scheme 3). The IR spectra of these compounds showed strong carbonyl absorption at 1,653–1,655 cm^{-1}. Condensation of the thiazolopyridazines derivative **19** with isocyanates and isothiocyanates afforded the corresponding carbamoyl (**20a**, **b**) and thiocarbamoyl (**21a–c**) derivatives respectively. However, condensation of the 4-sulfonamidophenylthiazolopyridazine derivative **22** with the appropriate isocyanate and isothiocyanate in dry acetone yielded the corresponding benzenesulfonyl urea (**23a–c**) and thiourea (**24a–c**) derivatives respectively. The IR spectra of these compounds exhibited two bands at 1,341–1,354 cm^{-1} and 1,148–1,158 cm^{-1}due to SO_2N group as well as a urea carbonyl band at 1,664–1,668 cm^{-1} in case of **23** and a thiourea carbonyl absorption at 1,125–1,130 cm^{-1}in **24**.

Biological evaluation

In vitro antibacterial and antifungal activities

The results revealed that 25 out of the tested 49 compounds displayed broad spectrum of antibacterial activity, with greater inhibitory effect on the growth of the tested

Scheme 2 Synthesis of compounds **10–15**

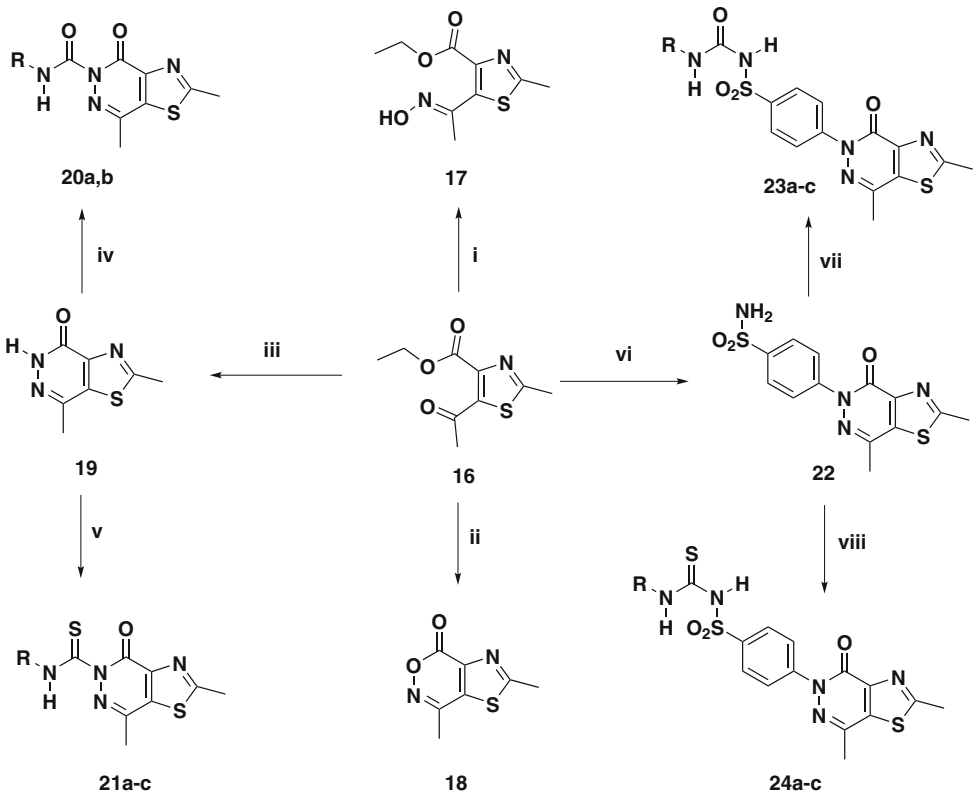


10: R = **a:** cyclo-C_6H_{11}, **b:** C_6H_5, **c:** 4-Cl-C_6H_4
11: R = **a:** CH_3, **b:** C_6H_5, **c:** 4-Cl-C_6H_4, **d:** 4-F-C_6H_4, **e:** COC_6H_5, **f:** 4-CH_3-C_6H_4
12: R = **a:** CH_3, **b:** C_6H_5, **c:** 4-Cl-C_6H_4, **d:** 4-F-C_6H_4
13: R = **a:** CH_3, **b:** C_6H_5, **c:** 4-Cl-C_6H_4, **d:** 4-F-C_6H_4, **e:** COC_6H_5
14: R = **a:** C_6H_5, **b:** 4-F-C_6H_4
15: R = **a:** C_6H_5, **b:** 4-F-C_6H_4, **e:** 4-CH_3-C_6H_4

Reagents and reaction conditions:
i: RNCO, reflux; **ii:** RNCS, reflux; **iii:** phenacyl bromide, reflux; **iv:** ethyl bromoacetate; **v:** diethyl oxalate, reflux; **vi:** 1,2-diiodoethane, reflux.

Gram positive strains compared to Gram negative ones. Fourteen compounds were able to exert mild to moderate antifungal activity against *C. albicans,* whereas, all the tested compounds lacked activity against *A. niger*. Compound **11d** was equipotent to Ampicillin (MIC 6.25 μg/ml) against *S. aureus*, whereas the analogs **4c**, **4d**, **10c**, **11c**, **13d** and **24c** (MIC 12.5 μg/ml) were 50 % less active than Ampicillin. Further, **4b**, **7**, **12d**, **13c**, **20b**, **21b**, **21c**, **22** and **24b** (MIC 25 μg/ml) showed 25 % of the activity of Ampicillin against the same microorganism. However, **13d** and **24c**, was as active as Ampicillin (MIC 12.5 μg/ml) against *B. subtilis*, and analogs **4d**, **10c**, **11d** and **21c** (MIC 25 μg/ml) displayed half the potency of Ampicillin. Three analogs namely **11d**, **13d** and **21c**, were able to produce noticeable growth inhibitory activity against *E. coli* (MIC 12.5 μg/ml) which represents half the activity of Ampicillin (MIC 6.25 μg/ml), whereas **4d**, **11c**, **13c**, **21b** and **24c** (MIC 25 μg/ml) exhibited moderate activity against the same organism when compared with Ampicillin. Additionally, the tested *P. aeruginosa* strain revealed moderate to weak activity towards some of the active compounds (MIC range 50–100 μg/ml). The antifungal activity of the tested compounds revealed that fourteen analogs were able to produce appreciable growth inhibitory activity against *C. albicans* (MIC values 12.5–100 μg/ml, respectively) comparable to that of Clotrimazole (MIC 6.25 μg/ml). Among these, **11d** and **24c** possessed half the activity of Clotrimazole, while the analogs **11c**, **13d** and **24b** showed 25 % of the standard against the same organism (Table 3).

Scheme 3 Synthesis of compounds **17–24**



20: R = **a:** C_6H_5, **b:** 4-Cl-C_6H_4
21: R = **a:** CH_3, **b:** C_6H_5, **c:** 4-Cl-C_6H_4
23: R = **a:** C_6H_5, **b:** 4-Cl-C_6H_4, **c:** cyclo-C_6H_{11}
24: R = **a:** CH_3, **b:** C_6H_5, **c:** 4-Cl-C_6H_4

Reagents and reaction conditions:
i: $NH_2OH.HCl$, sodium acetate, reflux; **ii:** $NH_2OH.HCl$, pyridine, reflux; **iii:** $NH_2NH_2.H_2O$;
iv: RNCO, reflux; **v:** RNCS, reflux; **vi:** 4-sulfamylphenylhydrazine.HCl, reflux; **vii:** RNCO, reflux;
viii: RNCS, reflux.

Structure–Activity Relationship (SAR)

A close examination of the structures of the active compounds revealed that most of the tested isoxazolo[4,5-*d*]pyridazines displayed better antimicrobial profile than their corresponding thiazolo[4,5-*d*]pyridazine congeners as evidenced by their MIC values recorded in Table 3. Among the isoxazolo[4,5-*d*]pyridazine series, the introduction of a substituted carboxamide/carbothioamide or trifluoroacetyl moieties at position-5 significantly altered the activity. In this view, the carboxamide derivatives **3b** and **3c** (R = C_6H_5 and 4-Cl–C_6H_4, respectively) showed slight improvement in the activity against the Gram positive *S. aureus* and *B. subtilis* (MIC values 50–100 μg/ml). Isosteric replacement of the carboxamide group with a carbothioamide one as in **4a–d** greatly enhanced the activity, and their antimicrobial potency and spectrum may be linked to the type and nature of the substituent (R). A significant broad spectrum antibacterial activity against the tested Gram positive, Gram negative bacteria (MIC values 12.5–100 μg/ml), and moderate antifungal activity towards *C. albicans* (MIC 50 μg/ml) were displayed by the analogs **4b–d** substituted with aromatic moieties (R = C_6H_5, 4-Cl–C_6H_4 and 4-F–C_6H_4, respectively). The decreasing trend in the activity was in the order **4d** > **4c** > **4b**. Compound **4a** (R = CH_3) was devoid of any antimicrobial activity. Moreover, the trifluoroacetyl analog **5** showed a noticeable improvement in the activity against the tested Gram positive bacterial strains (MIC 50 μg/ml). However, cyclization of **5** to the corresponding isoxazolo[4,5-*d*][1,2,4]triazolo[4,3-b]pyridazine (**6**), led to an observable reduction in the overall antimicrobial activity. Additionally, substitution of the N^5 with a benzenesulfonamide counterpart as in **7**, led to a significant enhancement in antibacterial spectrum and potential, as well as the antifungal activity (Scheme 1).

In Scheme 2, derivatization of the sulfonamide functionality into a substituted sulfonylurea moiety (**10c**; R = 4-Cl–C_6H_4) gave rise to a remarkable improvement in

the anti-Gram positive activity against *S. aureus* and *B. subtilis* (MIC values 12.5 and 25 μg/ml). Interestingly, the synthesis of their substituted sulfonylthioureido congeners (**11a–f**) resulted in a marked increase in both the antibacterial spectrum and potential, together with a moderate enhancement in the antifungal activity. Among these, the analogs **11c** and **11d** (R = 4-Cl–C_6H_4 and 4-F–C_6H_4, respectively) showed the highest activity, especially **11d** (R = 4-F–C_6H_4), which was found to be equipotent to Ampicillin against *S. aureus* (MIC 6.25 μg/ml). Further annulations of the substituted sulfonylthiourea functionality of compounds **11a–f** afforded various substituted thaizole derivatives **12–15**, with moderate antimicrobial potential and spectrum, and significantly high activity against the Gram positive strains (Table 3). The substituted thaizolidinones **13a–e** revealed the best antibacterial and antifungal activities, among which the analogs **13c** and **13d** (R = 4-Cl–C_6H_4 and 4-F–C_6H_4, respectively), displayed the most distinctive antimicrobial profile. Compound **13d** proved to be equipotent to Ampicillin against *B. subtilis* (MIC 12.5 μg/ml), whereas its activity against *S. aureus* and *E.Coli* was 50 % less than Ampicillin (MIC 12.5 *vs* 6.25 μg/ml, respectively). In addition, it showed moderate antifungal activity towards *C. albicans* which was about 25 % of Clotrimazole (MIC 25 *vs* 6.25 μg/ml, respectively).

Replacement of the isoxazole ring with the structurally related thiazole nucleus as in the thiazolo[4,5-*d*]pyridazine series **19–24** (Scheme 3) did not offer any advantage to the anticipated antimicrobial potential and spectrum. In general, the most prominent antimicrobial activity was confined to compounds comprising carboxamido/carbothioamido (**20a–b** and **21a–c** respectively) and the benzenesulfonylureido/thioureido (**23a–c** and **24a–c** respectively) functionalities. Accordingly, the phenylcarboxamido derivative **20b** (R = 4-Cl–C_6H_4) showed moderate activity against *S. aureus*, *B. subtilis* and *E. coli* (MIC values 25, 50 and 50 μg/ml respectively) whereas, its carbothioamido congener **21b** (R = C_6H_5) showed better activity against *E. coli* (MIC 25 μg/ml), in addition to a weak antifungal activity against the *C. albicans*. Introduction of a chlorine atom in the phenyl ring as in **21c** (R = 4-Cl–C_6H_4) resulted in a marked improvement in the overall antimicrobial spectrum especially towards *B. subtilis* and *E. coli* (MIC values 25 and 25 μg/ml, respectively), in addition to a mild antifungal potential against *C. albicans* (MIC 50 μg/ml). With regard to the benzenesulfonylureido/thioureido series (**23a–c** and **24a–c**), the type of function and the nature of N^1-substitution are markedly governing their antimicrobial potential. In this context, despite a very weak activity shown by compounds with aliphatic substituents (**23c** and **24a**; R = cyclo-C_6H_{11} and CH_3, respectively), the aryl- substituted analogs revealed a better spectrum and potential of antimicrobial activity. In particular, the 4-chlorophenylsulfonylthioureido derivative **24c** (R = 4-Cl–C_6H_4) featured the best antimicrobial activity among the thiazolo[4,5-*d*]pyridazine series, as it proved to be equipotent to Ampicillin against *B. subtilis* (MIC 12.5 μg/ml), and possessed 50 % of the activity of Ampicillin against *S. aureus* (MIC 12.5 μg/ml). Meanwhile, its activity against *E.Coli* was 25 % of that of Ampicillin (MIC 25 μg/ml), in addition to a moderate antifungal activity towards *C. albicans*, which was about 50 % of that of Clotrimazole (MIC 12.5 μg/ml, respectively).

Conclusions

In conclusion, the results revealed that 25 out of the tested 49 compounds displayed broad spectrum of antibacterial activity, with greater inhibitory effect on the growth of the tested Gram positive strains compared to Gram negative ones. Fouteen compounds were able to produce appreciable growth inhibitory activity against *C. albicans* when compared with the standard antifungal agent Clotrimazole. However, all of the tested compounds lacked antifungal activity against *Aspergillus niger*. Structurally, most of the tested isoxazolo[4,5-*d*]pyridazines displayed better antimicrobial profile than their corresponding thiazolo[4,5-*d*]pyridazine congeners, as evidenced by their MIC values. Compounds **11c**, **d**, **13d** and **24c** could be considered as the most active antimicrobial members identified in the present study. Finally, the biological results of such type of fused heterocycles may assist in further modification in order to optimize the anticipated chemotherapeutic activities, and make them the appropriate candidates for future development through modification or derivatization in order to design more potent and selective antimicrobial agents.

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