Synthesis and Biological Evaluation of New Fused Isoxazolo[4,5-d] Pyridazine Derivatives

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Three new heterocyclic ring systems, isoxazolo[4,5-d]-1,2,4-triazolopyridazines **12-15**, tetrazolo-[4,3-b]pyridazine **18** and isoxazolo[4,5-d]pyridazino[2,3-c]2H-triazines **16**,**17** along with isoxazolo[4,5-d]pyridazines **2**, **5-10** have been synthesized. Preliminary screening of these tricyclic heterocycles revealed that some of them possess significant antibacterial and antifungal activity.

Keywords: Isoxazoles; Triazoles; Pyridazines.

INTRODUCTION

Isoxazoles,[1-6] triazoles[7-10] and pyridazines[11-16] are biologically important heterocyclic compounds. Some of these compounds have antibacterial activity better than carbenicillin against Gram-negative strains[17] others are used as antimicrobial agents.[18,19] A variety of condensed triazolo[20-23] and triazino[7,22,24] derivatives show a wide range of pharmacological and biological properties. Prompted by these observations and in continuation to our work on the synthesis of biologically active heterocyclic compounds,[25-29] it was felt interesting to synthesize fused heterocyclic systems incorporating isoxazole, triazole and pyridazine moieties and evaluate their antimicrobial activity.

RESULTS AND DISCUSSION

The readily available ethyl 5-acetyl-3-methylisoxazole-4-carboxylate **1** served as starting material for the synthesis (Scheme I). Reaction of **1** with hydrazine derivatives afforded the 1-substituted 3,6-dimethyl-7-oxoisoxazolo-[4,5-d]pyridazines **2a,b**, while reaction with hydroxylamine hydrochloride **1** gave oxime **3** or 3,6-dimethyl-7-oxoisoxazolo[4,5-d]-1,2-oxazine **4** depending on the reaction conditions (see Experimental). Consistent with the assigned structure the ^{1}H NMR spectrum of oxime **3** exhibited a triplet and quartet at δ 1.40 and 4.33 for the CH_3 and CH_2 of the ester group, respectively. These signals are missing in the ^{1}H NMR spectrum of the oxazine derivative **4**. The ^{13}C NMR data of compounds **2** and **4** are summarized in Table 2 and the chemical shift values conform to the suggested structures.

The MS of the pyridazinone **2a** showed a molecular ion as the base peak at *m/z* 165 while for **2b** the base peak was the [M-$NHSO_2C_6H_4NCO$] species and the molecular ion was only 25%. On the other hand, the mass spectrum of oxazine derivative **4** exhibited the molecular ion peak at *m/z* 166 while the base peak is at *m/z* 41.

Condensation of the pyridazine derivative **2a** with isocyanates and isothiocyanates afforded the corresponding carbamoyl **5a-c** and thiocarbamoyl **6a-c** derivatives respectively. The IR spectra of the carbamoyl derivatives **5** exhibited two distinguishable carbonyl absorptions at 1668-1672 cm^{-1} and 1654-1659 cm^{-1} whereas compounds **6a-c** revealed distinct thiocarbonyl band at 1060-1150 cm^{-1} as well as the pyridazinone carbonyl band at 1655-1658 cm^{-1}. The ^{13}C NMR data of compounds **5** and **6** as summarized in Table 2 is in agreement with the suggested structures.

Refluxing of **2a** with phosphorus oxychloride gave the 4-chloro derivative **7**, which on reaction with ethanolic hydrazine hydrate at room temperature yielded the 3,6-dimethyl-7-hydrazinoisoxazolo[4,5-d]pyridazine **8** in 86% yield. Reaction of **7** with thiourea gave the thiol derivative **9**, which on treatment with an aqueous solution of sodium hydrogen sulfate and sodium nitrite yielded the disulfide **10**. In accordance with the suggested structure, the ^{13}C

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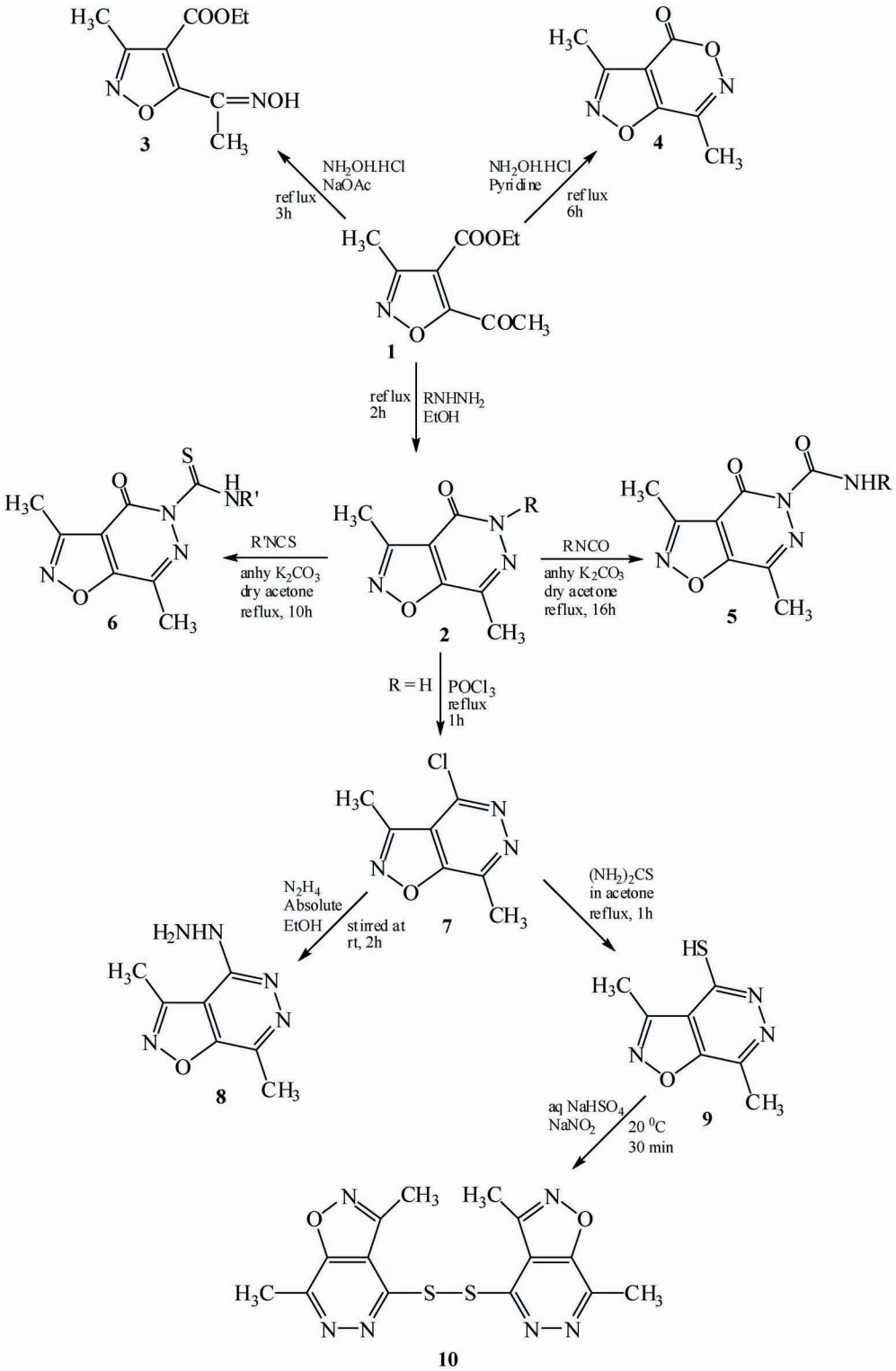
Scheme I



NMR spectrum of **9** exhibited the expected number of signals for the aromatic carbons as well as two methyl signals at δ 10.48 and 15.82 (Table 2).

Reaction of the hydrazino derivative **8** with acid chlo-

Table 1. Physical and analytical data

Compound	R or R′	Yield (%)	M.P (°C)	Formula	Analysis							
					Calcd. %				Found %			
					C	H	N	S	C	H	N	S
2a	H	82	257	$C_7H_7N_3O_2$	50.91	4.27	25.44		50.95	4.30	25.60	
2b	*p*-$NH_2SO_2C_6H_4$	88	176	$C_{13}H_{12}N_4O_4S$	48.74	3.77	14.49	10.01	48.82	3.88	14.52	10.21
3		70	58	$C_9H_{12}N_2O_4$	50.94	5.70	13.20		51.00	5.90	13.31	
4		65	212	$C_7H_6N_2O_3$	50.61	3.64	16.86		50.75	3.80	16.78	
5a	Cyclohexyl	82	220	$C_{14}H_{18}N_4O_3$	57.92	6.25	19.30		58.02	6.36	19.42	
5b	C_6H_5	85	228	$C_{14}H_{12}N_4O_3$	59.15	4.25	19.71		59.26	4.37	19.90	
5c	*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	
6a	C_6H_5	80	186	$C_{14}H_{12}N_4O_2S$	55.99	4.03	18.65	10.67	56.00	4.12	18.70	10.81
6b	*p*-ClC_6H_4	78	204	$C_{14}H_{11}ClN_4O_2S$	50.33	3.31	16.73	9.57	50.30	3.42	16.84	9.68
6c	*p*-FC_6H_4	76	162	$C_{14}H_{11}FN_4O_2S$	52.82	3.48	17.60	10.07	52.92	3.53	17.72	10.14
7		84	102	$C_7H_6ClN_3O$	45.78	3.29	22.88		45.87	3.30	22.00	
8		86	160	$C_7H_9N_5O$	46.92	5.06	39.08		46.91	5.13	39.20	
9		78	230	$C_7H_7N_3OS$	46.39	3.89	23.19	17.69	46.40	3.92	23.25	17.71
10		82	258	$C_{14}H_{12}N_6O_2S_2$	46.65	3.35	23.32	17.79	46.62	3.42	23.30	17.82
11a	CH_3	76	145	$C_9H_{12}ClN_5O_2$	41.94	4.69	27.17		41.02	4.72	27.25	
11b	*p*-$CH_3OC_6H_4$	82	156	$C_{15}H_{16}ClN_5O_3$	51.50	4.61	20.02		51.65	4.72	20.15	
12	CH_3	70	245	$C_9H_9N_5O$	53.19	4.46	34.46		53.21	4.56	34.56	
13	*p*-$CH_3OC_6H_4$	74	170	$C_{15}H_{13}N_5O_2$	61.00	4.43	23.71		60.99	4.52	23.86	
14	CH_2Br	75	236	$C_9H_8BrN_5O$	38.31	2.86	24.82		38.19	2.90	24.76	
15	COOEt	72	305	$C_{11}H_{11}N_5O_3$	61.10	5.13	32.39		61.21	5.23	32.40	
16		66	>300	$C_9H_7N_5O_3$	46.35	3.02	30.03		46.38	3.10	30.15	
17a	C_6H_5	78	246	$C_{15}H_{13}N_5O$	64.50	4.69	25.07		64.62	4.72	25.18	
17b	CH_3	64	230	$C_{10}H_{11}N_5O$	55.29	5.10	32.23		55.31	5.23	32.30	
18		62	207	$C_7H_6N_6O$	44.21	3.18	44.19		44.30	3.22	44.20	

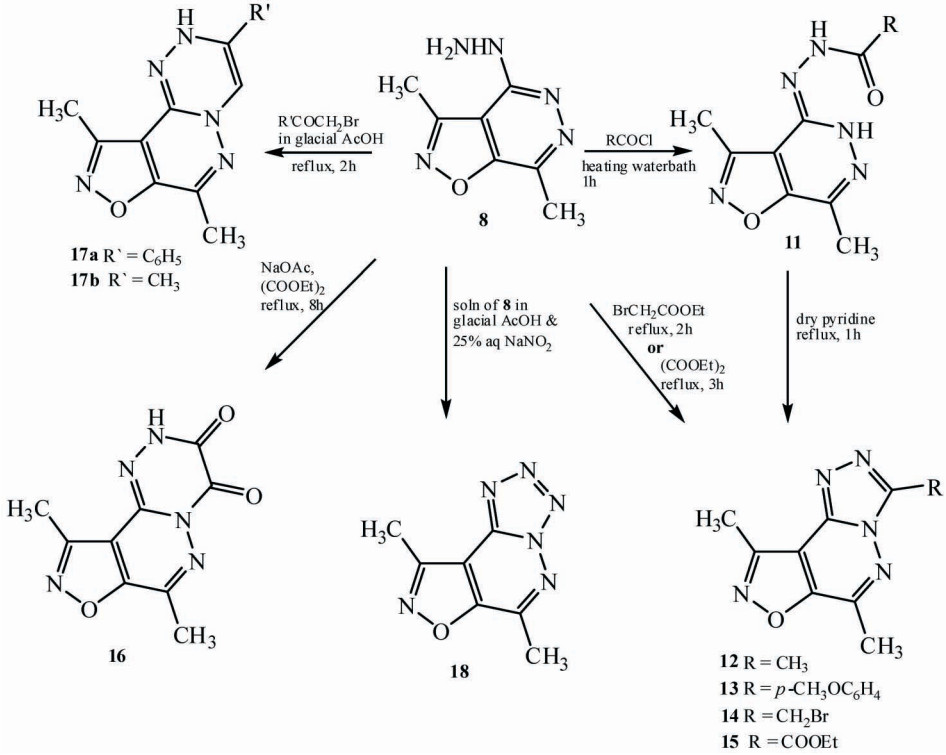
Table 2. ^{13}C NMR spectral data of isoxazolo[4,5-d]pyridazine derivatives

Compd	Aromatic C	CH_3 and other C	CO & CS
2a	133.12, 156.75, 158.63	15.64, 14.61	163.77
2b	107.81, 111.52, 125.41, 130.67, 134.28	12.74, 14.90	
	145.95, 158.73, 160.52		168.51
4	115.32, 134.20, 154.82, 158.93	11.02, 15.83	170.20
5a	112.37, 132.39, 155.95, 159.57	9.09, 14.61	168.89, 178.20
		23.29, 24.15, 32.45, 46.34 (cyclohexyl C)	
5b	111.83, 121.23, 124.72, 127.78, 128.20, 134.68, 149.26, 158.73	10.89, 15.06	169.33, 180.12
6a	109.71, 121.38, 124.81, 127.09, 129.05, 136.28, 152.48, 158.42	10.55, 14.59	167.85, 205 (CS)
6b	110.35, 122.36, 124.10, 125.99, 133.42, 147.09, 156.28, 159.36	12.02, 15.13	168.85, 200.20 (CS)
7	114.82, 135.06, 155.20, 157.43, 162.03	10.56, 15.48	
9	113.80, 136.55, 146.70, 155.04, 158.36	10.48, 15.82	
12	114.46, 133.25, 144.78, 152.68, 156.70, 158.45	11.21, 13.03, 15.68	
15	113.66, 134.02, 146.60, 154.28, 155.89, 159.32	11.34, 13.74, 18.10, 60.54 (CH_3)	170.33
16	113.86, 132.98, 148,33, 152.98, 158.72	10.78, 15.90	168.78, 170.50
17b	112.98, 132.56, 136.42, 138.05, 146.35, 157.56, 162.78	10.66, 13.35, 15.84	
18	113.63, 133.42, 145.32, 156.78, 158.14	10.47, 15.82	

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

rides afforded the corresponding acyl hyrazides hydrochlorides **11** which readily cyclized in hot pyridine to the 3-substituted 6,9-dimethylisoxazolo[4,5-d]l,2,4-triazolo-[4,3-b]pyridazines **12** and **13** (Scheme II). The IR spectra

Scheme II



of the acyl hydrazides **11** exhibited secondary carbonyl absorption at 1662-1672 cm^{-1}, which is missing in the IR spectra of the cyclic derivatives, **12** and **13**.

Cyclocondensation of **8** with ethyl bromoacetate gave the 3-(bromomethyl)-1,2,4-triazolo derivative **14**. Its ^{1}H NMR exhibited a singlet of two protons intensity at δ 4.78 for the CH_2 as well as two methyl singlets at δ 2.47 and 2.85.

In 1969 Polts *et al*[30] reported that the reaction of an excess of oxalic acid with 1-hydrazinophthalazine at 160 °C to give a 50% yield of s-triazolo[3,4-a]phthalazine, presumably arising from the decarboxylation of the originally formed s-triazolo[3,4-a]phthalazine-3-carboxylic acid. Zimmer *et al*[31] isolated the 2H-*as*-triazolo[3,4-a]phthalazine-3,4-dione in 90% yield instead of the expected ethyl s-triazolo[3,4-a]phthalazine-3-carboxylate from the reaction of hydralazine with excess ethyl oxalate. However, in our case, when we refluxed **8** with ethyl oxalate, the product isolated was ethyl 6,9-dimethylisoxazolo[4,5-d]-1,2,4-triazolo[4,3-b]pyridazine-3-carboxylate **15** or 7,10-dimethylisoxazolo[4,5-d]pyridazino[2,3-c]-2H-l,2,4-triazine-3,4-dione **16** depending on the reaction conditions (see Experimental) as evidenced by analytical and spectral data (Tables 1 & 2).

Moreover, refluxing the hydrazino compound **8** with β-bromoketones in acetic acid afforded the corresponding 3-substituted 7,10-dimethylisoxazolo[4,5-d]pyridazino-[2,3-c]-2H-1,2,4-triazines **17a**,**b**. The ^{1}H NMR spectra of the triazines **17a** and **17b** showed, besides the CH_3 and aromatic protons, an exchangeable NH signal at δ 11.95-12.46. The structure of **17a** was further confirmed by its MS analyses. A molecular ion peak at *m/z* 279 was detected, while the base peak is at *m/z* 162.

Finally, when a solution of **8** in acetic acid was allowed to react with sodium nitrite the product was found to be the tetrazolo derivative **18**, a reaction similar to what's reported by Gatta *et al*.[23]

ANTIMICROBIAL ACTIVITY

Antimicrobial testing of compounds **2-8**, **12-15** and **17** was carried out (Table 3) and it was found that compounds **5a**, **6c** and **17a** showed maximum activity (+++) (MIC = 25 μg/mL) against *S. aureus,* compounds **5c**, **6b** and **14** showed moderate activity (++) (MIC = 50 μg/mL), while compounds **5b**, **7, 8** and **17b** exhibited feeble activity (+) (MIC = 75 μg/mL). Compounds **6c** and **17a** showed the maximum activity against Gram-negative strain *E. coli*, while **5c**, **6b** and **14** exhibited moderate activity and com-

Table 3. Antimicrobial activities of synthesized compounds (+++ for maximum activity, MIC[a] = 25; ++ for moderate activity, MIC = 50; + for slight activity MIC = 75; and – for inactive compounds)

Organism	2a	2b	3	4	5a	5b	5c	6a	6b	6c	7	8	12	13	14	15	17a	17b
S aureus (Gram-positive)	-	-	-	-	+++	+	++		++	+++	+	+	-	-	++	-	+++	+
E. coli (Gram-negative)	-	-	-	-	+	-	++		++	+++	+	-	-	-	++	-	+++	-
C. albicans (Fungi)	+	+	+++	-	-	-	-		-	-	+	+	-	-	++	-	++	++

[a] Minimum inhibitory concentration (MIC in μg/mL)

pounds **5a** and **7** showed weak activity. In the antifungal activity profile against *C. albicans* compound **3** revealed maximum inhibition (+++), compounds **14**, **17a** and **17b** showed moderate inhibition (++) and compounds **2a**, **2b**, **7** and **8** were slightly active (+). However, other compounds were found inactive towards various strains of bacteria. Quantitative assays were done on active compounds only.

EXPERIMENTAL

Melting points were determined on Gallenkamp melting point apparatus and are uncorrected. IR spectra were obtained on Perkin-Elmer 297 infrared spectrophotometer using potassium bromide pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DPX-400-FT spectrometer using TMS as internal standard. MS were obtained on a Kratos MS 30. Elemental analyses were performed by Microanalyses unit, King Abdulaziz University, Jeddah, Saudi Arabia. Ethyl 5-acetyl-3-methylisoxazole-4-carboxylate was obtained from Maybridge Chemical Company Limited, UK.

1-Substituted-3,6-dimethyl-7-oxoisoxazolo[4,5-d]pyridazines (2a,b)

A solution of ethyl 5-acetyl-3-methylisoxazole-4-carboxylate **1** (0.39 g, 0.002 mol) in ethanol (25 mL) was refluxed with the appropriate hydrazine for 2 h. The pyridazines which separated after concentration of the reaction mixture were filtered, washed with cold ethanol, and recrystallized from ethanol as needles.

2a: IR (KBr, ν_{max}, cm^{-1}) 1662 (CO), 3285 (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).

2b: IR (KBr, ν_{max}, cm^{-1}) 1665 (CO), 3337 and 3200 (NH_2); ^{1}H 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 (3)

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}) 1735 (CO), 3310 (OH); ^{1}H 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).

3,6-Dimethyl-7-oxoisoxazolo[4,5-d]-1,2-oxazine (4)

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}) 1750 (CO); ^{1}H 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).

1-Substituted-carbamoyl-3,6-dimethyl-7-oxoisoxazolo-[4,5-d]pyridazines (5a-c)

A mixture of the pyridazine derivative **2a** (0.91 g, 0.005 mol), anhydrous K_2CO_3 (0.01 mol) in dry acetone (50 mL) was stirred. A solution of the appropriate isocyanate (0.005 mol) 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 sepa-

rated upon acidification with dilute HCl, which was filtered and recrystallized from ethanol.

5a: IR (KBr, ν_{max}, cm^{-1}) 1654 (CO), 1668 (CO), 3289 (NH); ^1H NMR (DMSO-d_6) δ 0.99-1.92 (m, 11H, cyclohexyl H), δ 2.46 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 10.23 (s, 1H, NH).

5b: IR (KBr, ν_{max}, cm^{-1}) 1656 (CO), 1670 (CO), 3275 (NH); ^1H 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).

5c: IR (KBr, ν_{max}, cm^{-1}) 1655 (CO), 1672 (CO), 3282 (NH); ^1H 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).

1-Substituted-thiocarbamoyl-3,6-dimethyl-7-oxoisoxazolo[4,5-d]pyridazines (6a-c)

A mixture of the pyridazine derivative **2a** (0.91 g, 0.005 mol), anhydrous K_2CO_3 (0.01 mol) in dry acetone (50 mL) was stirred. A solution of the appropriate isocyanate (0.005 mol) 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 with dilute HCl. The precipitated crude product was filtered and recrystallized from ethanol.

6a: IR (KBr, ν_{max}, cm^{-1}) 1655 (CO), 1060 (CS), 3290 (NH); ^1H NMR (DMSO-d_6) δ 2.42 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 7.12-7.35 (m, 5H, C_6H_5), 10.22 (s, 1H, NH).

6b: IR (KBr, ν_{max}, cm^{-1}) 1657 (CO), 1150 (CS), 3268 (NH); ^1H NMR (DMSO-d_6) δ 2.41 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 7.00-7.45 (m, 4H, ArH), 9.88 (s, 1H, NH).

6c: IR (KBr, ν_{max}, cm^{-1}) 1658 (CO), 1078 (CS), 3288 (NH).

7-Chloro-3,6-dimethylisoxazolo[4,5-d]pyridazine (7)

Compound **2a** (0.83 g, 0.005 mol) was suspended in phosphorus oxychloride (10 mL) and the mixture was refluxed for 1 h. The excess of phosphorus oxychloride was distilled under reduced pressure and the residual syrup was poured onto finely crushed ice (~50 g), extracted with ether and the solvent was removed to give pale yellow solid of the chloro derivative **7** which was recrystallized from methanol as needles; ^1H NMR ($CDCl_3$) δ 2.44 (s, 3H, CH_3), 2.60 (s, 3H, CH_3).

3,6-Dimethyl-7-hydrazinoisoxazolo[4,5-d]pyridazine (8)

A solution of the 4-chloro derivative **7** (0.84 g, 0.005 mol) and 94% hydrazine hydrate (1 g) in absolute ethanol (50 mL) was stirred at room temperature for 2 h. The solid which was separated during stirring was filtered and crystallized from methanol as needles; IR (KBr, ν_{max}, cm^{-1}) 3192 and 3342 ($NHNH_2$), ^1H NMR (DMSO-d_6) δ 8.65-9.25 (broad s, 3H, NH and NH_2), 2.39 (s, 3H, CH_3), 2.68 (s, 3H, CH_3).

3,6-Dimethylisoxazolo[4,5-d]pyridazine-7-thiol (9)

A solution of **7** (0.84 g, 0.005 mol) in acetone (25 mL) was refluxed with thiourea (0.76 g, 0.010 mol) for 1 h. The reaction was then cooled to 0 °C. A solution of 0.5 M Na_2CO_3 (10 mL) was added slowly while keeping the temperature below 5 °C. The mixture was stirred overnight. The resulting reaction mixture was neutralized with 5% HCl (v/v). The precipitated product was collected, washed with water, and recrystallized from DMSO as pale yellow needles; IR (KBr, ν_{max}, cm^{-1}) 2610 (SH); ^1H NMR (DMSO-d_6) δ 2.54 (s, 3H, CH_3), 2.60 (s, 3H, CH_3).

Bis(3,6-dimthylisoxazolo[4,5-d]pyridazin-7-yl)disulfide (10)

To a suspension of **9** (0.36 g, 0.002 mol) and $NaHSO_4$ (2 g) in water (15 mL), $NaNO_2$ (2 g) was gradually added (30 min) with stirring at 20 °C. The disulfide which separated was recrystallized from DMSO as pale yellow needles; ^1H NMR (DMSO-d_6) δ 2.54 (s, 3H, CH_3), 2.70 (s, 3H, CH_3).

3-Substituted-6,9-dimethylisoxazolo[4,5-d]-1,2,4-triazolo[4,3-b]-pyridazines (12) and (13)

The hydrazino derivative **8** (0.45 g, 0.0025 mol) was heated on a water bath with the appropriate acid chloride (0.003 mol) for 1 h. The acylhydrazides **11** that separated out in the form of hydrochlorides were recrystallized from ethanol as needles (Table 1). Cyclization to the triazolo derivatives **12** and **13** was performed by refluxing these acylhydrazides (0.001 mol) in dry pyridine (8 mL) for one hour. The reaction mixture was then poured onto 10% Na_2CO_3 solution and the separated solid was recrystallized from methanol as needles.

11a; IR (KBr, ν_{max}, cm^{-1}) 1668 (CO), 3270 (NH).

11b; IR (KBr, ν_{max}, cm^{-1}) 1562 (CO), 3265 (NH).

12; ^1H NMR (DMSO-d_6): δ 2.48 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 2.68 (s, 3H, CH_3).

13; ^1H NMR (DMSO-d_6): δ 2.44 (s, 3H, CH_3), 2.80 (s, 3H, CH_3), 7.42-7.72 (m, 5H, C_6H_5).

3-Bromomethyl-6,9-dimethylisoxazolo[4,5-d]-1,2,4-triazolo[4,3-b]-pyridazines (14)

A solution of **8** (0.45 g, 0.0025 mol) in ethanol (20 mL) was refluxed with ethyl bromoacetate (0.43 g, 0.0026

mol) for 2 h. The reaction mixture was then concentrated to yield white solid which was recrystallized from ethanol as needles; ^{1}H NMR (DMSO-d_6) δ 2.42 (s, 3H, CH_3), 2.88 (s, 3H, CH_3), 4.78 (s, 2H, CH_2).

Ethyl 6,9-dimethylisoxazolo[4,5-d]-1,2,4-triazolo[4,3-b]pyridazine-3-carboxylate (15)

A mixture of **8** (0.45 g, 0.0025 mol) and excess of ethyl oxalate (10 mL) was refluxed for 3 h. The reaction mixture was cooled to room temperature. A solid was separated which was collected and recrystallized from DMF as pale yellow needles; IR (KBr, ν_{max}, cm^{-1}) 1725 (CO); ^{1}H NMR (DMSO-d_6) δ 1.45 (t, J = 6 Hz, 3H, CH_3), 2.50 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 4.42 (q, J = 6 Hz, 2H, CH_2).

7,10-Dimethylisoxazolo[4,5-d]pyridazino[2,3-c]-2H-l,2,4-triazine-3,4-dione (16)

A mixture of **8** (0.45 g, 0.0025 mol), sodium acetate (1.0 g) and ethyl oxalate (0.38 g, 0.0027 mol) in ethanol (30 mL) was refluxed for 8 h. The reaction mixture was then poured into ice-cold water. The precipitated solid was recrystallized from DMF as needles; IR (KBr, ν_{max}, cm^{-1}) 1692 (CO), 1740 (CO), 3182 (NH); ^{1}H NMR (DMSO-d_6) δ 2.52 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 8.48 (broad s, 1H, NH).

3-Substituted-7,10-dimethylisoxazolo[4,5-d]pyridazino-[2,3-c]-2H-l,2,4-triazines (17)

A solution of **8** (0.45 g, 0.0025 mol) in glacial acetic acid (10 mL) was refluxed with the appropriate β-bromoketone (0.0026 mol) for 2 h. After bringing it to room temperature it was poured over ice cold water. The separated solid was collected and recrystallized from ethanol as needles.

17a (R′ = C_6H_5); ^{1}H NMR (DMSO-d_6): δ 2.46 (s, 3H, CH_3), 2.80 (s, 3H, CH_3), 7.38-7.58 (m, 5H, C_6H_5), 12.64 (s, 1H, NH); MS *m/z* (relative intensity) 279 (M^+, 30), 278 (M-H, 15), 264 (M-CH_3, 12), 251 (M-N_2, 4), 202 (11), 187 (4), 173 (8), 162 (100), 148 (20), 109 (22), 93 (10), 96 (8), 81 (21), 66 (22).

17b (R′ = CH_3); ^{1}H NMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 7.01 (s, 1H, CH), 11.95 (s, 1H, NH).

6,9-Dimethylisoxazolo[4,5-d]-1,2,3,4-tetrazolo[4,3-b]-pyridazine (18)

A stirred solution of **8** (0.45 g, 0.0025 mol) in glacial acetic acid (10 mL) was gradually treated with 25% aqueous $NaNO_2$ (15 mL) at room temperature. The tetrazolo derivative which separated as white solid was recrystallized from ethanol as needles. ^{1}H NMR ($CDCl_3$): δ 2.50 (s, 3H, CH_3), 2.70 (s, 3H, CH_3).

BIOLOGICAL TESTING

Compounds **2-8**, **12-16** and **17** were screened for their antibacterial and antifungal activity following agar-diffusion method[32] using Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli*. The antifungal testing was carried out against *Candida albicans*.

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