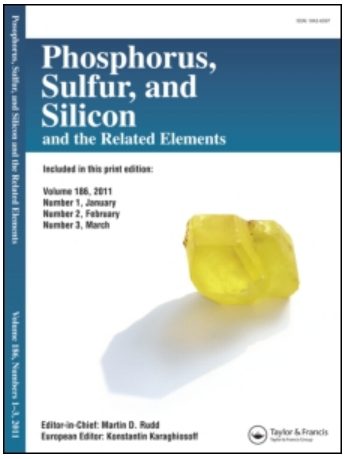

Phosphorus, Sulfur, and Silicon and the Related Elements

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Synthesis and Antibacterial Activity of Substituted Thiosemicarbazides and of 1,3,4-Thiadiazole or 1,2,4-Triazole Derivatives

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Synthesis and Antibacterial Activity of Substituted Thiosemicarbazides and of 1,3,4-Thiadiazole or 1,2,4-Triazole Derivatives

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*The synthesized series of new thiosemicarbazide derivatives (**1**, **6–10**) in reactions with carbon disulphide produced, according to the reaction conditions, the dithioacids (**4**, **30**) or the 5-substituted 1,3,4-thiadiazolo-2-thiol derivatives (**2**, **27**). The dithioacids were cyclized, in the reaction with hydrazine, into the 4-ami-no-1,2,4-triazolo-2-thiol derivatives (**5**, **31**). One of these compounds (**31**) was transformed into the 1,2,4-triazolo-1,3,4-thiadiazine derivative (**33**). The compounds **6–9** were also exposed to the condensation with aldehydes. 4-phenylpiperazinocarbothiohydrazide (**6**) was exposed to the action of isothiocyanates, which gave the compounds **16–20**, and these cyclized to the 1,3,4-thiadiazoloamino derivatives (**21–23**).*

*The susceptibility of aerobic and anaerobic bacteria to some of the new derivatives were tested. The anaerobes were the most susceptible at concentrations in ranges less than 6.2 to 100 μg/mL to derivative: **9** (64% were susceptible), **1**, **13** (for 60%), and **7** (for 56%).*

Keywords 1,3,4-thiadiazole; 1,2,4-triazole; 1,2,4-triazolo-1,3,4-thiadiazine; thiosemicarbazides; antibacterial activity

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INTRODUCTION

Many thiosemicarbazide derivatives described in the chemical literature produced evidence of the antibacterial, antimalarial,[1] antituberculitic,[3] as well as anticancer[2] activity. The compounds obtained from these heterocyclic systems appeared to be of varied biological activity as well. The 1,3,4-thiadiazole system-containing derivatives showed antitubercu-litic,[4,5] antibacterial, antimycotic,[6,7] and analgesic[8] actions. The 1,2,4-triazolo system-con-taining compounds appeared to be antimycotic,[9] Alzheimer's-disease retarding,[10] and icterus-Type C-virus-inhibiting[11] agents. Their influence over the immunology of HIV-1 virus infections[12] was proved as well. However, the 1,2,4-triazolo-1,3,4-thiadiazine deri-vatives were recognized as antiparasitic[13] substances. Thus, further syntheses and investigation of this group of compounds seems to be well founded. Syntheses of the new thiosemicarbazide derivatives, as well as of amino-substituted azol derivatives, are reported in this article.

RESULTS AND DISCUSSION

In continuation of our search for potentially antibacterial substances, the new thiosemi-carbazides and the products of their cyclization reactions with carbon disulphide to 1,3,4-thiadiazole, 1,2,4-triazole, and 1,2,4-triazolo-1,3,4-thiadiazine derivatives were obtained.

The substrate for further syntheses was 4-methyl-4-phenyl-3-thiosemicarbazide **1**, obtained by carbon disulphide addition to N-methylaniline, followed by methylphenylthio-carbaminoylsulphonyl-acetic acid and thioacetic acid elimination by a hydrazine hydrate action.[14]

The products of the carbon disulphide addition to compound **1** depended upon the reaction conditions. At the elevated temperature, 5-N-methyl-N-phenyl-1,3,4-thiadiazolo-2-thiol **2** was obtained, which on a methyl iodide action gave its S-methyl derivative **3**. At r.t., however, dithioacid **4** was obtained. The reaction of compound **4** with hydrazine resulted in a 4-amino-5-N-methyl-N-phenyl-1,2,4-triazolo-2-thiol **5** formation. From compound **1**, the dithiocarbazoic acid methyl ester **15** was obtained as well, but an attempt at obtaining from this compound the 1,3,4-thiadiazole substituted with amine groups and then the morpholine derivative of this thiadiazole failed, only compound **2** was obtained instead.

The action of corresponding amines on compound **1** produced 4-N-substituted thiosemicarbazide derivatives (**6–10**). Three of them were condensed with 5-nitrofuryl aldehyde, which gave the condensation products (**12–14**).

The thiosemicarbazide derivative **6** was also obtained by a hydrazine hydrate action upon 4-phenylpiperazin-1-carbothiomethyl ester **11**, which was produced in the reaction of 1-phenylpiperazine with carbon disulphide and methyl iodide in the presence of triethylamine. This method, however, was less effective (Scheme 1).

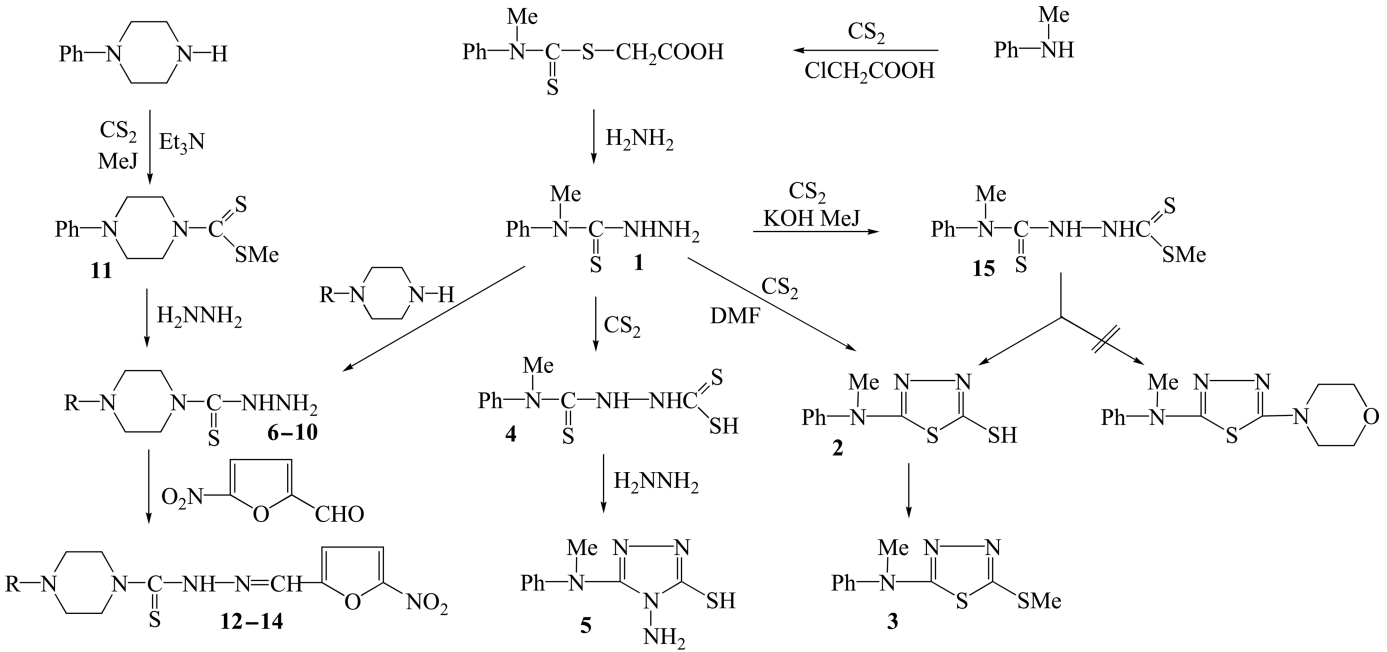
At the next stage of work, the derivatives of 4-phenylpiperazin-1-carbothiohydrazide **6** were obtained. As in the 4-phenyl-4-methyl-3-thiosemicarbazide case, the products of the carbon disulphide addition were dependent upon the conditions. The corresponding dithio-acid **30**, or 1,3,4-thiadiazolo-2-thiol **27**, was produced. From compound **27**, the S-methyl derivative **28** was obtained, and this one, when heated with morpholine, gave derivative **29**. The same compound was obtained as well, when the dithioacid **30** was refluxed in an excess of morpholine. The potassium salt of compound **30**, necessary for further syntheses, was obtained by a carbon disulphide addition to **6** in the presence of potassium hydroxide. From compound **30**, potassium salt the derivative **27** was obtained by microwave irradiation. The same potassium salt was transformed, under the hydrazine hydrate influence, into the 4-N-amino derivative of 1,2,4-triazolo-2-thiol **31**. This last compound was produced by two methods: either by several hours of heating of **30b** salt in ethanol or by several minutes of action of microwave in radiation. Both methods gave good yields. From compound **31** the condensate **32** with p-chlorobenzoic aldehyde was obtained. With regard to the communication,[13] 1,2,4-triazolo-1,3,4-thiazine derivatives have been recognized as antiparasitic agents; the phenylpiperazine derivative of this condensed system (**33**) was synthesised by phenacyl bromide action upon the compound **31**.

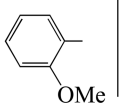
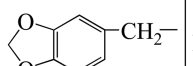
4-phenylpiperazin-1-carbothiohydrazine **6** was also exposed to the action of several isothiocyanates, which gave the corresponding thiocarbamino derivatives (**16–20**). Some of them were cyclized in boiling ethanol to the corresponding 2,5-diamino-1,3,4-thiadiazole derivatives (**21–23**).

In the condensation of **6** with salicylaldehyde, p-methoxybenzaldehyde, and 3,4,5-tri-methoxybenzaldehyde, the products **24–26** were obtained. The choice of aldehydes was dictated by the good results obtained earlier of the antibacterial investigations (Scheme 2). The physical data are summarized in Table I.

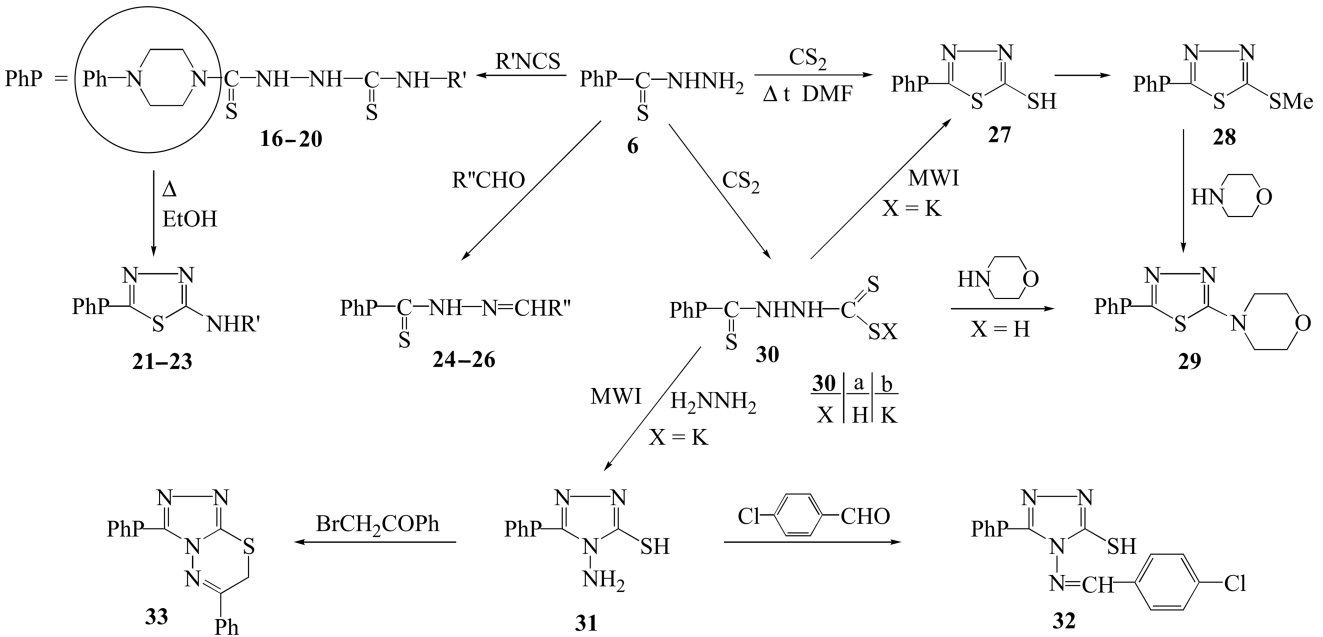
MICROBILOGICAL ACTIVITY

The investigations included 25 strains of anaerobic bacteria (see Table II) and 25 strains of aerobic bacteria (*Staphylococcus aureus*,



No	6	7, 12	8, 13	9, 14	10
R					HO–(CH2)2–

SCHEME 1



No	16	17	18, 21	19, 22	20, 23
R'	CH_2=$CHCH_2$–	p–Cl–Ph–	Ph–	p–Br–Ph–	2,4–$(Cl)_2$–Ph

No	24	25	26
R"	O–HO–Ph–	p–MeO–Ph–	3,4,5$(MeO)_3$Ph–

SCHEME 2

TABLE I Characteristics of the Newly Synthesized Compound

Compound no.	M.P. [°C] Solvent for Crystallization	Yield (%)	Formula	IR (KBr) (cm^{-1})	^1H NMR δ (ppm)
2	151 Methanol	95	$C_9H_9N_3S_2$	719, 1061, 1286, 1348, 1492, 1546, 2754, 2895, 3069, 3447	$CDCl_3$: 3.42 (s, 3H, NCH_3); 7.2–7.5 (m, 5H, Ph); 11.1 (s, 1H, NH)
3	124–125 Methanol	15	$C_{10}H_{11}N_3S_2$	704, 1058, 1240, 1490, 1650, 2760, 2998, 3209, 3270	DMSO-d_6: 2.64 (s, 3H, SCH_3); 3.56 (s, 3H, NCH_3); 7.2–7.5 (3m, 5H, Ph)
4	103–104 Etanol	73	$C_9H_{11}N_3S_3$	563, 703, 1068, 1359, 1496, 2927, 3258	
5	116–117 Ethanol	14	$C_9H_{11}N_5S$	702, 1014, 1142, 1287, 1491, 1639, 2962, 3206, 3273	DMSO-d_6: 3.40 (s, 3H, NCH_3); 4.50 (s, 2H, NH_2); 7.15–7.50 (m, 5H, Ph); 8.70 (s, 1H, NH)
6	176–177 Dioxan	55	$C_{11}H_{16}N_4S$	686, 801, 1018, 1226, 1425, 1502, 1551, 1600, 2361, 2836, 1961, 3221, 3265	DMSO-d_6: 3.10; 3.85 (2m, 8H, CH_2 piperazine); 4.80 (s, 2H, NH_2); 6.70–7.30 (3m, 5H, Ph); 9.2 (s, 1H, NH)
7	151–152 Methanol	34	$C_{12}H_{18}ON_4S$	737, 1033, 1239, 1502, 2832, 2914, 3238	DMSO-d_6: 2.98 (s, 4H, $2CH_2$); 3.78 (s, 3H, OCH_3); 3.8 (s, 4H, $2CH_2$); 4.76 (s, 2H, NH_2); 6.8–7.0 (2m, 4H, Ph); 9.15 (s, 1H, NH)
8	173–174 Methanol	47	$C_{10}H_{15}N_5S$	702, 1232, 1485, 1598, 2849, 2918, 3217, 3437	DMSO-d_6: 3.5 and 3.85 (2m, 8H piperazine); 5.8 (s, 2H, NH_2); 6.65 and 6.8 and 7.55 and 8.15 (4m, 4H pyridine); 9.15 (s, 1H, NH)
9	152–153 Methanol	46	$C_{13}H_{18}O_2N_4S$	701, 1038, 1227, 1500, 2824, 2854, 2906, 2952, 3228	DMSO-d_6: 2.3 (s, 2H, CH_2); 3.4 and 3.7 (2m, 8H piperazine); 5.98 (s, 2H, CH_2); 6.7–7.5 (3m, 3H, Ph)

10	143–144 Methanol	44	$C_7H_{16}ON_4S$	703, 1482, 1600, 2852, 3220, 3342	DMSO-d_6: 2.35 (m, 4H, $2CH_2$); 3.4–3.7 (2m, 8H piperazine); 4.40 (t, 2H, OH); 4.70 (s, 2H, NH_2); 9.05 (s, 1H, NH)
11	71–72 Ethanol	61	$C_{12}H_{16}N_2S_2$	696, 765, 936, 1011, 1153, 1276, 1425, 1494, 1597, 2363, 2807	$CDCl_3$: 2.69 (s, 3H, CH_3); 3.32; 4.35 (2m, 8H, CH_2 piperazine); 6.99–7.32 (2m, 5H, Ph)
12	158–160 Ethanol	24	$C_{17}H_{19}O_4N_5S$	759, 963, 1019, 1233, 1309, 1480, 1560, 2906, 3213, 3436	DMSO-d_6: 3.07; 4.07 (2m, 8H piperazine); 6.88–7.00 (2m, 4H, Ph); 7.18 and 7.77 (2d, 2H furyl); 8.07 (s, 1H, CH); 11.65 (s, 1H, NH)
13	101–103 Ethanol	22	$C_{15}H_{16}O_3N_6S$	776, 1030, 1292, 1471, 1506, 2830, 3072, 3147	DMSO-d_6: 3.67 and 4.02 (2m, 8H piperazine); 6.64 and 6.82 and 7.58 and 8.70 (t, d, t, d, 4H pyridine); 7.19 and 7.79 (2d, 2H furyl); 8.09 (s, 1H, CH); 11.67 (s, 1H, NH)
14	155–157 Ethanol	38	$C_{18}H_{19}O_5N_5S$	809, 1037, 1242, 1354, 1486, 2804, 2901, 3294	DMSO-d_6: 3.44; 3.89 (2m, 8H piperazine); 3.61 (s, 2H, CH_2); 7.15 and 7.77 (2d, 2H furyl); 7.22–7.42 (2m, 3H, Ph); 8.04 (s, 1H, CH); 11.56 (s, 1H, NH)
15	108 Methanol	67	$C_{10}H_{13}N_3S_3$	702, 968, 1068, 1360, 1512, 2942, 3274, 3452	$CDCl_3$: 2.61 (s, 3H, SCH_3); 3.68 (s, 3H, NCH_3); 7.3–7.65 (2m, 5H, Ph); 9.6 (s, 2H, 2NH)
16	155 Methanol	51	$C_{15}H_{21}N_5S_2$	693, 931, 1021, 1223, 1502, 1557, 2831, 3173	DMSO-d_6: 3.33 and 3.97 (2m, 8H piperazine); 3.86 (m, 1H, CH); 4.11 (s, 2H, CH_2); 5.05 and 5.20 (2d, 2H, CH_2); 6.77–7.24 (3m, 5H, Ph); 8.08–9.59 (3s, 3H, NH)
17	215–216 Methanol	58	$C_{18}H_{20}N_5S_2Cl$	759, 1021, 1226, 1493, 1532, 1599, 2922, 3186	DMSO-d_6: 3.26; 3.47 (2m, 8H piperazine); 7.20–7.55 (3m, 9H, Ph); 9.99; 10.28 (2s, 3H, NH)
18	151–153 Ethanol	54	$C_{18}H_{21}N_5S_2$	694, 935, 1024, 1250, 1501, 1601, 2361, 2838, 2992, 3166	DMSO-d_6: 3.25; 4.01 (2m, 8H piperazine); 6.75–7.44 (3m, 10H, Ph); 9.48–9.83 (3s, 3H, NH)

(Continued on next page)

TABLE I Characteristics of the Newly Synthesized Compound (*Continued*)

Compound no.	M.P.[°C] Solvent for Crystallization	Yield (%)	Formula	IR (KBr) (cm^{-1})	^1H NMR δ (ppm)
19	147–149 Ethanol	62	$C_{18}H_{20}N_5S_2Br$	694, 759, 929, 1022, 1225, 1492, 1599, 2361, 2921, 3190	DMSO-d_6: 3.25; 4.01 (2m, 8H piperazine); 6.75–7.54 (4m, 9H, Ph); 9.62–9.80 (2s, 3H, NH)
20	126–127 Cyclohexan	41	$C_{18}H_{19}N_5S_2Cl_2$	693, 753, 813, 1020, 1226, 1301, 1505, 1599, 2361, 2923, 3156, 3368	DMSO-d_6: 3.22–3.99 (2m, 8H piperazine); 6.75–7.66 (5m, 8H, Ph); 9.41–9.77 (2s, 3H, NH)
21	185–186 Methanol	57	$C_{18}H_{19}N_5S$	755, 1229, 1319, 1375, 1445, 1489, 1601, 2848, 3194, 3245, 3438	$CDCl_3$: 3.31; 3.61 (2m, 8H piperazine); 6.88–7.36 (4m, 10H, Ph)
22	212–214 Ethanol	41	$C_{18}H_{18}N_5SBr$	755, 938, 1030, 1233, 1440, 1493, 2361, 2825, 3258, 3431	$CDCl_3$: 3.23; 3.64 (2m, 8H piperazine); 6.83–7.44 (4m, 9H, Ph)
23	162–163 Ethanol	81	$C_{18}H_{17}N_5SCl_2$	694, 761, 934, 1229, 1381, 1441, 1493, 1594, 2970, 3207, 3404	$CDCl_3$: 3.31; 3.66 (2m, 8H piperazine); 6.89–7.37 (5m, 8H, Ph)
24	207–209 Ethanol	52	$C_{18}H_{19}ON_4S$	749, 1019, 1236, 1303, 1535, 2361, 2856, 3235, 3431	DMSO-d_6: 3.25; 4.10 (2m, 8H, CH_2 piperazine); 3.26 (s, 1H, OH); 6.78–7.42 (5m, 9H, Ph); 8.48 (s, 1H, CH); 11.55 (s, 1H, NH)
25	184–186 Ethanol	67	$C_{19}H_{22}ON_4S$	1019, 1170, 1250, 1509, 1602, 2361, 2834, 3175, 3436	DMSO-d_6: 3.26; 4.05 (2m, 8H, CH_2 piperazine); 3.79 (s, 3H, OCH_3); 6.77–7.61 (4m, 9H, Ph); 8.10 (s, 1H, CH); 11.14 (s, 1H, NH)
26	206–208 Methanol	70	$C_{21}H_{26}O_3N_4S$	759, 1128, 1234, 1328, 1504, 1557, 2361, 2934, 3156, 3399	DMSO-d_6: 3.72; 4.04 (2m, 8H, CH_2 piperazine); 3.68 (s, 3H, OCH_3); 3.81 (s, 6H, OCH_3); 6.76–7.27 (3m, 7H, Ph); 8.03 (s, 1H, CH); 11.32 (s, 1H, NH)

27	189–190 Ethanol	56	$C_{12}H_{14}N_2S_3$	718, 761, 936, 1056, 1230, 1501, 1551, 2361, 2919, 3094	DMSO-d_6: 3.29; 3.42 (2m, 8H, CH_2 piperazine); 6.78–7.28 (3m, 5H, Ph); 1360 (s, 1H, NH)
28	111–112 Ethanol	75	$C_{13}H_{16}N_4S_2$	696, 760, 932, 1160, 1227, 1507, 1596, 2847, 3001, 3435	$CDCl_3$: 2.70 (s, 3H, SCH_3); 3.3 and 3.65 (2m, 8H piperazine); 6.9–7.4 (2m, 5H, Ph)
29	176–177 Ethanol	51	$C_{16}H_{21}ON_5S$	752, 1111, 1236, 1494, 1600, 2856, 2968, 3434	DMSO-d_6: 3.30–3.40 (2m, 8H morpholine); 3.60 and 3.80 (2m, 8H piperazine); 6.90–7.30 (3m, 5H, Ph)
30a	161–162 Methanol	59	$C_{12}H_{16}N_4S_3$	686, 751, 931, 1018, 1226, 1425, 1503, 1551, 1600, 2836, 3220, 3265	DMSO-d_6: 3.15; 3.90 (2m, 8H, CH_2 piperazine); 4.80 (sz.s, 2H, NH_2); 6.75–7.30 (3m, 5H, Ph); 9.20 (s, 1H, NH)
30b	207–208	90	$C_{12}H_{15}N_4S_3K$	913, 1230, 1367, 1501, 1599, 2360, 2834	
31	177–178 Methanol	60	$C_{12}H_{16}N_6S$	719, 936, 1057, 1230, 1381, 1501, 1551, 2815, 2929, 3095, 3456	DMSO-d_6: 3.15; 3.85 (2m, 8H piperazine); 5.00 (s, 2H, NH_2); 6.70–7.30 (3m, 5H, Ph); 9.10 (s, 1H, NH)
32	160–161 Ethanol	63	$C_{19}H_{19}SCl$	720, 934, 1242, 1509, 1600, 2862, 3158	DMSO-d_6: 3.20 and 3.40 (2m, 8H piperazine); 6.80–7.70 (5m, 9H, 2Ph); 8.16 (s, 1H, CH); 13.62 (s, 1H, NH)
33	115–116 Methanol	43	$C_{20}H_{20}N_6S$	691, 761, 933, 1191, 1231, 1449, 1518, 1597, 1676, 2827, 3430	$CDCl_3$: 3.31; 3.66 (2m, 8H piperazine); 6.91–8.03 (4m, 10H, Ph)

TABLE II The Minimal Inhibitory Concentration (MIC) for the Compounds

No.	Anaerobic Bacteria	Number of Strains	MIC (μg/mL)																															
			Metronidazole (+)				Compounds **1** (+) **2** (*)						Compounds **3** (+) **4** (*)						Compounds **5** (+) **6** (*)						Compounds **7** (+) **8** (*)					
			≥200	25–100	12,5	≤6,2	≥200	100	50	25	12,5	≤6,2	≥200	100	50	25	12,5	≤6,2	≥200	100	50	25	12,5	≤6,2	≥200	100	50	25	12,5	≤6,2
	Gram-positive																																	
1	Peptococcus niger	1				+	*		+				+ *						*					+				*			+
2	Peptostreptococcus magnus	2				++	+ *	+ *					++ **						++ **						**		+		+			
3	Peptostreptococcus micros	2				++		+	+ *		*		*	+ *			+		+ *	*		+						+	+ *	*
4	Actinomyces israelii	1				+	*		+				+ *						*	+				+	+ *							
5	Actinomyces naeslundii	1				+	+ *						+ *						+ *						+ *							
6	Propionibacterium granulosum	2	++				**	+				+	++ **						+	+ *					+	+	*			*		
	Gram-negative																																	
7	Prevotella bivia	1				+	+			*			*	+					+ *						+			*			+		
8	Prevotella buccalis	2				++	+ **			+			+ **	+					+	+	**				+ *		+ *					
9	Prevotella intermedia	2			+	+	+ **	+					++ **						+ **	+					+ **			+					
10	Prevotella loescheii	1				+	*					+	+ *						+	*								+ *					
11	Porphyromonas asaccharolytica	2			+	+	+ **		+				+ **	+					++ **						+ *			*			+		
12	Fusobacterium nucleatum	1				+		+		*			*			+			+ *									+ *					
13	Fusobacterium necrophorum	2			+	+	+ **		+				+ **	+					+ *	*			+		+ *			+	*				
14	Bacteroides forsythus	3			+	++	++ ***		+				+++ ***						++ **	*	+				+++ **	*							
15	Bacteroides ureolyticus	2	+			+	+ **	+					++ **						++ *		*				+ **	+							

TABLE II The Minimal Inhibitory Concentration (MIC) for the Compounds (*Continued*)

	MIC (μg/ml)																																	
	Compounds **9** (+) **10** (*)						Compounds **12** (+) **13** (*)						Compounds **14** (+) **15** (*)						Compounds **16** (+) **17** (*)						Compounds **27** (+) **28** (*)						Compounds **29** (+) **32** (*)					
No.	≥200	100	50	25	12,5	≤6, 2	≥200	100	50	25	12,5	≤6, 2	≥200	100	50	25	12,5	≤6, 2	≥200	100	50	25	12,5	≤6, 2	≥200	100	50	25	12,5	≤6, 2	≥200	100	50	25	12,5	≤6, 2
1			*			+	*			+			*			+			+					*			*			+			+	*		
2	*	+	+ *				++ *					*	**		+	+			+ **		+				+ **		+				++ **					
3	*			++		*	+	*		+		*	*			*		+	++ **						*	+			*	+		*		*		
4	+ *						+		*				+ *						+		*				*		+				+ *					
5	+ *						+ *						+ *						+ *						+ *						+ *					
6			++ **				**	+				+	++ **						++ *					*	+ **			+			++ **					
7					+		+					*	*					+	+ *						+ *						+			*		
8	+	+	**				+		*	*	+		+ **			+			+ **			+			**	+		+			++ **					
9	+ **	+					++ *		*				+ **	+					++ **						++ **						++ **					
10			*	+			*			+			+ *						+					*			*		+		+			*		
11	+ *		*	+			+ *				*	+	+ **	+					++ *	*					+ **					+	++ *		*			
12	*			+					+	*						+					+				*			+			+				*	
13	+ *			+		*	+	*	+	*			+ **				+		++ **						+ **		+				++ *		*			
14	+ **	++	*				++ **		**			+	++ ***			+			+++ **		*				++ ***				+		+++ ***					
15	++ **						+ *		+ *				+ **	+					++ **						++ **						++ **					

Corynebacterium spp., *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Pseudomonas stutzeri*). The aerobic bacteria and anaerobic bacteria were isolated from patients with infections of the oral cavity, respiratory tract, and abdominal cavity.

The susceptibility (MIC) anaerobes were determined by means of a plate dilution technique in Brucella agar supplemented with 5% of sheep blood,[15,16] whereas aerobes were determined in Mueller-Hinton agar. The incubation was performed at 37°C for 48 h in anaerobic or aerobic conditions for 24 h. MIC was regarded as the lowest thiosemicarbazide derivative concentration inhibiting the growth of bacteria.

The derivatives tested exhibited differential activity against anaerobic bacteria. The anaerobes were the most susceptible at concentrations in ranges for $\leq$6.2 to 100 μg/mL to derivative: **9** (64% were susceptible), **1**, **13** (for 60%) and **7** (56%).

The anaerobes were much less susceptible to the following derivatives: **4** (for 96% strains MIC $\geq$200 μg/mL); **29**, **15** (for 92% MIC $\geq$200 μg/mL); **16** (for 88% MIC $\geq$200 μg/mL).

The aerobic bacteria were generally not susceptible to concentrations of the derivatives studied, with the exeption of derivative **10**, which was active against 52% of strains at concentrations in ranges of 50–100 μg/mL.

The derivatives had activities comparable to those of metronidazole against anaerobic bacteria and comparable to those of amikacin against aerobes (Table II).

EXPERIMENTAL

Melting points were determined with the Mettler FP-2 apparatus and are uncorrected. The IR spectra were taken with Satellite FTIR spectrophotometer. The ^{1}H NMR spectra were taken with a Varian Gemini 200 BS-487c spectrometer. The microwave Plazmotronika (100 W, 500 MHz wave frequency) reactor was used for the syntheses of two compounds.

5-N-Methyl-N-Phenyl-1,3,4-Thiadiazolo-2-Thiol (2)

4-phenyl-4-methyl-3-thiosemicarbazide (0.91 g, 5 mmole), DMF (3 mL), and CS_2 (1.5 mL) were refluxed for 4 h. Then ice water was added until the product precipitated, which was collected and recrystallized.

2-Methylsulphonyl-5-N-Methyl-N-Phenyl-1,3,4-Thiadiazole (3)

To KOH (0.085 g, 1.5 mmole) dissolved in ethanol (5 mL), compound **2** (0.22 g, 1 mmole) was added and, when dissolved, was treated with methyl iodide (0.1 mL, 1.5 mmole). The whole was refluxed for 0.5 h. On cooling the precipitated product was collected and washed on the filter with cold ethanol.

4-Phenyl-4-Methyl-1-Carbodithiosemicarbazide Acid (4)

To KOH (1.1 g, 20 mmole) dissolved in water (5 mL) and ethanol (20 mL), 4-phenyl-4-methyl-3-thiosemicarbazide (1.81 g, 10 mmole) was added and, when dissolved, was treated with CS_2 (2 mL). The solution was stirred for 1.5 h. On acidifying the mixture with acetic acid, compound **4** precipitated.

4-Amino-5-N-Methyl-N-Phenyl-1,2,4-Triazolo-2-Thiol (5)

The mixture of compound **4** (0.26 g, 1 mmole), ethanol (5 mL), and 98% hydrazine hydrate (0.15 mL, 3 mmole) was refluxed for 2 h. On cooling, compound **5** precipitated and was filtered.

4-N-Substituted-1-Carbothioic Acid Hydrazide (6–10)

A. Compound **1** (1.81 g, 10 mmole) dissolved in hot acetonitrile (10 mL) was treated with the appropriated amine (10 mmole). The mixture was refluxed for 0.5 h. The mixture was cooled then the precipitate was filtered and recrystallized.
B. Compound **11** (2 g, 8 mmole) dissolved in ethanol (20 ml) was treated with 98%-hydrazine hydrate and refluxed for 16 h. On cooling the precipitated compound **6** was filtered, washed thoroughly with water and recrystallized.

4-Phenyl-Piperazin-1-Dithiocarbazoic Acid Methyl Ester (11)

4-phenylpiperazine (1.5 mL, 10 mmole) dissolved in ethanol (10 mL) was treated with triethylamine (1.4 mL, 10 mmole) and carbon disulphide (0.75 mL, 12.5 mmole). The precipitate formed was dissolved with water, then methyl iodide (0.63 mL, 10 mmole) was added. The mixture was stirred for 1 h. The precipitate of compound **11** was filtered and recrystallized.

The Condensates of 4-N-Substituted Thiosemicarbazides With 5-Nitrofuryl Aldehyde (12–14)

The compound **7**, **8**, or **9** (10 mmole), respectively, was dissolved in hot ethanol (10 mL) and treated with a stoichiometric amount of 5 nitrofuryl aldehyde. The mixture was refluxed for 5 min. and then allowed to stand at an ambient temperature for 12 h. The precipitated compounds **12–14**, respectively, were collected and washed on the filter with ethanol.

Dithiocarbazoic Acid Methyl Ester (15)

To KOH (0.56 g, 10 mmole) dissolved in water (3 mL) and ethanol (10 mL), 4-phenyl-4-methyl-3-thiosemicarbazide (1.8 g, 10 mmole) was added, and, when dissolved, it was treated with carbon disulphide (0.64 mL, 10 mmole). The mixture was stirred for 15 min., then methyl iodide (0.63 mL, 10 mmole) was added and stirred for another 15 min. On cooling the product precipitated.

Thiocarbamino Derivatives (16–20)

Compound **6** (0.6 g, 2.5 mmole) dissolved in hot ethanol (10 mL) was treated with a stoichiometric amount of the appropriate isothiocyanate and refluxed for 10 min. The products precipitated either while hot or on cooling.

1,3,4-Thiadiazolo-2-Ylo-Amines (21–23)

Compounds **18–20** (1 mmole), respectively, dissolved in ethanol (15 mL) were refluxed for 6 h. On cooling, the precipitate of compounds (**21–23**), respectively, were filtered and recrystallized.

The Condensates of 4-N-Phenyl-Piperazinothiosemicarbazide With Aldehydes (24–26)

Compound **6** (2.4 g, 10 mmole) dissolved in ethanol (10 mL) was treated with the corresponding benzaldehyde (10 mmole). The mixture was refluxed for 10 min. and allowed to stand at r.t. for 12 h. The precipitated compounds (**24–26**) were filtered and recrystallized.

5-(4-Phenyl-Piperazin-1-Ylo)-[1,3,4]-Thiadiazolo-2-Thiol (27)

A. The mixture of compound **6** (1.2 g, 5 mmole), dimethylformamide (3.0 mL), and carbon disulphide (1.5 mL) was refluxed for 4 h. Ice

water was added, and then the precipitated product was filtered and recrystallized.

B. Compound **30b** (1.75 g, 5 mmole) dissolved in dimethylsulphoxide (3 mL) was irradiated with microwaves (3 min., temp. 170–180°C). When the reaction was completed, the mixture was treated with ice (20 g) and acidified with acetic acid to pH 7. The precipitate of compound **27** was filtered and recrystallized.

2-Methylsulphanyl-5-(4-Phenyl-Piperazin-1-Ylo)-[1,3,4]-Thiadiazole (28)

To KOH (0.085 g, 1.5 mmole) dissolved in ethanol (5 mL), compound **27** (0.28 g, 1 mmole) was added and, when dissolved, was treated with methyl iodide (0.7 mL, 1.5 mmole). The mixture was stirred for 0.5 h. On cooling, the product precipitated.

5-(4-Phenylpiperazin)-2-Morpholino-1,3,4-Thiadiazole (29)

A. Compound **28** (0.3 g, 1 mmole) was refluxed with morpholine (3 mL) and dimethylformamide (2 mL) for 30 h. On cooling the mixture, the precipitated compound **29** was filtered.

B. Compound **30a** (0.78 g, 2.5 mmole) was refluxed with morpholine (2 mL) for 2 h. On cooling the mixture, the precipitated compound **29** was filtered.

N′-4-Phenyl-Piperazin-1-Carbothioyl)-Hydrazinodithiocarbazoic Acid (30a)

To KOH (1.1 g, 20 mmole) dissolved in water (5 mL) and ethanol (20 mL), compound **6** (2.4 g, 10 mmole) was added and, when dissolved, was treated with carbon disulphide (2 mL). The mixture was stirred for 1.5 h, and then acidified with acetic acid and cooled with ice; the precipitated compound **30a** was filtered.

N′-(4-Phenyl-Piperazin-1-Carbothioyl)-Hydrazinodithiocarbazoic Acid potassium Salt (30b)

To KOH (0.56 g, 10 mmole) dissolved in ethanol (20 mL), compound **6** (2.36 g, 10 mmole) was added and stirred for 5 min. Then carbon disulphide (1.2 mL, 20 mmole) was added and stirred for another 45 min. The reaction mixture was treated with anhydrous diethyl ether (20 mL), and then the precipitate of salt **30b** was filtered.

4-Amino-5-(4-Phenyl-Piperazin-1-Ylo)-4H-[1,2,4]-Triazolo-3-Thiol (31)

A. The mixture of compound **30b** (0.35 g, 1 mmole), ethanol (5 mL), and 98% hydrazine hydrate (0.1 mL, 2 mmole) was refluxed for 4 h. Water (20 mL) was added, and then neutralized with acetic acid. The precipitated compound **31** was filtered and recrystallized.
B. Compound **30b** (1.75 g, 5 mmole) was dissolved in dimethylsulphoxide (3 mL) and 98% hydrazine hydrate (0.24 mL, 5 mmole) was added. The mixture was irradiated with microwaves (3 min., temp. 170–180°C). Ice (20 g) was added, and then the mixture neutralized with acetic acid. The precipitate of compound **31** was collected and recrystallized.

The Condensate of 4-Amino-5-(4-Phenylpiperazin)-1,2,4-Triazolo-2-Thiol With P-Chloro-Benzoic Aldehyde (32)

Compound **31** (0.41 g, 1.5 mmole) was dissolved in hot ethanol (10 mL) and treated with p-chlorobenzoic aldehyde (0.21 g, 1.5 mmole). The mixture was refluxed for 15 min. Compound **31** precipitated while hot. On cooling with ice, the product was filtered.

6-Phenyl-3-(4-Phenyl-Piperazin-1-Ylo)-7H-[1,2,4]-Triazolo[3,4-b][1,3,4]thiazidine (33)

Compound **31** (0.69 g, 2.5 mmole) dissolved in hot absolute ethanol (10 mL) was treated with phenacyl bromide (0.5 g, 2.5 mmole). The whole was stirred under refux for 6 h. The reaction mixture was cooled to r.t. and neutralized with an aqueous solution of Na_2CO_3. The precipitate of compound **33** was filtered and recrystallized.

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