N-[4-(3*H*-1,3,4-Oxadiazoline-2-thion-5-yl)phenyl]-*N'*-substituted Thioureas: Synthesis and Antimicrobiological Activities

N-[4-(3*H*-1,3,4-Oxadiazolin-2-thion-5-yl)phenyl]-*N'*-substituierte Thioharnstoffe: Synthese und antibakterielle Aktivität

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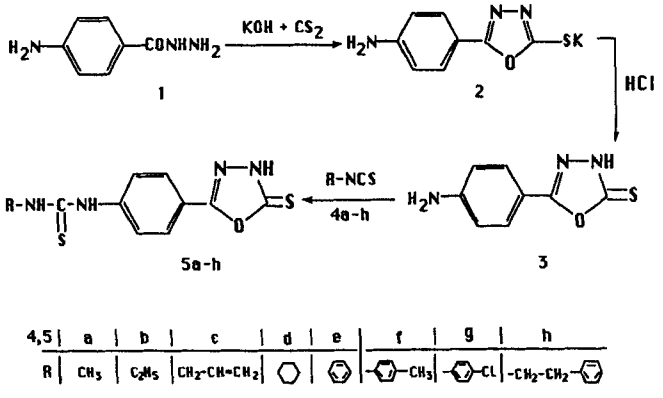
Diverse pharmacological properties are associated with 3*H*-1,3,4-oxadiazoline-2-thione derivatives. These include anticonvulsant[1-4] and fungicidal[5] properties. On the other hand thiourea derivatives possess antiphytoviral[6], virucidal[7], growth inhibitory[8-10], and herbicide[11] activities. All these data prompted us to combine these moieties into a single molecule. The compounds synthesized were tested for their antimicrobial activities against seven microorganisms.

The synthesis of *N'*-alkyl or aryl *N*-[4-(3*H*)-1,3,4-oxadiazoline-2-thion-5-yl)phenyl]-thiourea derivatives **5a-h** was achieved by refluxing 5-(4-aminophenyl)-3*H*-1,3,4-oxadiazoline-2-thion (**3**)[12] with appropriate alkyl or arylisothiocyanates **4a-h** in dioxane (Scheme).



Physical and some spectrophotometric data of **5a-h** are summarized in table 1.

Spectrometric data are in agreement with the expected structure. As an example IR-spectra of **5a-h** show besides characteristic absorptions bands, a peak at 1345 cm^{-1} corresponding to the C=S group[13]. No absorption band corresponding to the primary amine group of **3** was encountered. In the ^{1}H-NMR-spectra the NH signal of the thioamide group of 1,3,4-oxadiazole appeared at very low field at ~ 13.4-14.66 ppm range as a very broad peak. NH-peaks of the thiourea group were exhibited at ~ 10 ppm. They were exchangeable with D_2O. In their MS all compounds **5** show M^+ peaks losing the pertinent isothiocyanate molecule[14] to m/z = 193 (compound **3**).

Compounds **5a-h** were tested fort their *in vitro* antibacterial activities against some gram positive (*Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, *Sarcina lutea* ATCC 9341) and some gram negative (*Escherichia coli* ATCC 10536, *Proteus mirabilis* ATCC 14153, *Klebsiella pneumoniae* ATCC 4352) bacteria using the tube dilution method[15], in comparison with Penicillin G. The results are summarized in table 2. Highest activity was exhibited by the phenethyl derivative **5h**. None of the compounds was active against *P. mirabilis*. The compounds **5a**, **5f**, and **5g** do not show any antibacterial potency. Only compound **5h** was active against six bacterial stems, namely *S. areus*, *E. coli*, *K. pneumoniae*, *B. subtilis*, and *B. cereus* (MIC: 50 µg/ml), *S. lutea* (MIC: 3.1 µg/ml). Compounds **5b**, **5c**, **5d**, **5e**, and **5h** show inhibitory activity against *S. aureus* and *S. lutea* with MIC values between 3.1 and 100 µg/ml. Besides **5e** is active against *B. subtilis* and *B. cereus*; **5d** was also active against *B. cereus*. Penicillin G does not exhibit any inhibitory potency against these two bacteria.

Table 1: Physicl constants and selected IR data of **5a-h**

Comp. Nr.	Mp.(°C) Yield % (Recr.sol.)*	Mol.formula (Mol.Wt.)	Analysis calcd/found C	H	N	IR (KBr) cm^{-1} C=S**	C=S***
5a	223-225 75(a)	$C_{10}H_{10}N_4S_2O$ (266.35)	45.10 45.03	3.78 3.86	21.04 21.0	1348	1168
5b	200-201 75(a-b)	$C_{11}H_{12}N_4S_2O$ (280.37)	47.12 47.09	4.32 4.28	19.98 20.02	1345	1173
5c	169-170 73(c)	$C_{12}H_{12}N_4S_2O$ (292.38)	49.30 49.34	4.14 4.13	19.16 19.12	1349	1172
5d	215-216 42(a-b)	$C_{15}H_{18}N_4S_2O$ (334.46)	53.87 53.91	5.42 5.46	16.75 16.75	1342	1175
5e	158-159 73(c-b)	$C_{15}H_{12}N_4S_2O$ (328.42)	54.86 54.79	3.68 3.67	17.06 17.08	1345	1170
5f	173-174 45(a-b)	$C_{15}H_{11}ClN_4S_2O$ (362.86)	49.65 49.86	3.06 3.06	15.44 15.34	1343	1171
5g	163-165 76(c-b)	$C_{16}H_{14}N_4S_2O$ (342.44)	56.12 56.07	4.12 4.09	16.36 16.21	1346	1169
5h	160-161 84(c-b)	$C_{17}H_{16}N_4S_2O$ (356.47)	57.28 57.47	4.52 4.48	15.72 15.49	1346	1168

* a: methanol, b: H_2O, c: ethanol; ** thiourea; *** 1,3,4-oxadiazole-2-thione

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Table 2: *In vitro* antibacterial activity data (MIC) of **5a-h** (μg/ml)

	5a	5b	5c	5d	5e	5f	5g	5h	Penicillin G
S.aureus	-	50	100	25	100	-	-	50	2-6
S.lutea	-	50	50*	50*	50	-	-	3.1	2.1-4
P.mirabilis	-	-	-	-	-	-	-	-	8-13
E.coli	-	-	-	-	-	-	-	50*	64-450
K.pneumoniae	-	-	-	-	-	-	-	50*	128-245
B.subtilis	-	-	-	-	25	-	-	50	-
B.cereus	-	-	-	50	50	-	-	50	-

* MBC (μg/ml)

5c, 5d, 5h show bactericide activity against *S. lutea*, *E. coli*, *K. pneumoniae* with a MBC value of 50 μg/ml. It appeared that substituted phenyl rings or small substituents such as methyl group at the *N'*-position hinder antimicrobial activity.

By analogy to urea derivatives which decompose in solution[16], thioureas may also lead to isothiocyanates. As these compounds may exhibit some antibacterial, pesticide and poison gas activities[17], we checked the stability of **5a-h** in water and its mixtures with acetone at room temp. and at 37°C. Under these conditions no decomposition products were observed, using two TLC systems. Antimicrobial activity seems to be related to the molecular structure of the compounds.

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Experimental Part

M.P.: Büchi (Model Tottoli) apparatus, uncorr.- IR spectra (KBr): Perkin Elmer 1420.- ^{1}H-NMR spectra: Bruker WP 60 (60 MHz), Bruker WM 250 (250 MHz), and Bruker AC 300 (300 MHz), DMSO-d_6 and DMF-d_7 as solvents and TMS as internal standard.- Electron impact spectra: Finnigan Mat CH 7A; positive FAB mass spectra: Finnigan Mat CH5 DF.- Chromatographic separations: Chromatotron 7024 T (Harrison Research) using glass rotors with 4 mm layer of silicagel 60PF 254 containing gypsum.- Microanalyses: Perkin Elmer elemental analyzer 240C.

Synthesis of the compound **3**: lit.[12]

Preparation of thiourea dervatives **5a-h**

A mixture of 5-(4-aminophenyl)-3*H*-1,3,4-oxadiazoline-2-thione (**3**) (0.01 mol) and of the appropriate isothiocyanate **4a-h** (0.012 mol) in 30 ml of dioxane was refluxed for 4 h. After evaporation, the separated solid was filtered and purified by repetitive crystallisation from appropriate solvents. Compounds **5e**, **5f**, and **5g** were firstly purified by Chromatotron using chloroform-methanol (9:1), under NH_3, eluting unreacted amine **3** and impurities. After then elution was continued without NH_3 to give compounds **5** which were crystallized from solvents given in table 1.

Microbiology

In vitro antibacterial activity was determined by the tube dilution method[15]. The compounds were dissolved in acetone. Tow-fold dilutions of each compound were made in *Mueller-Hinton* liquid medium (concentration range 0.3-200 μg/ml). Each bacteria culture was incubated for 18 h at 37°C in nutrient broth. The cultures were then diluted to 1/100. 0.5 ml of these dilutions were inoculated in tubes with compound solutions. For each compound a control was included. The tests were carried out in triplicate. MIC or MBC values were calculated after 18 h of incubation at 37°C.

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