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Antibacterial Evaluation of Novel *N*-Arylimino-1,2,3-dithiazoles and *N*-Arylcyanothioformamides.

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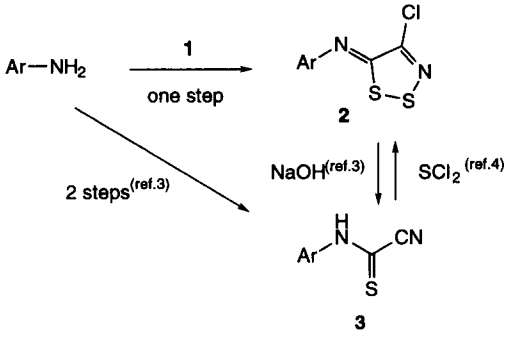
Abstract: *N*-Aryl-1,2,3-dithiazoles **2** and the corresponding *N*-arylcyanothioformamides **3** have been synthesized *via* 4,5-dichloro-1,2,3-dithiazole derivatives, and their antibacterial activity measured; the dithiazoles are significantly active against Gram-positive bacteria.

Studying the chemistry of the 4,5-dichloro-1,2,3-dithiazolium chloride **1** and its derivatives, we recently explored the synthesis of benzoxazin-4-ones, benzothiazin-4-ones and *N*-arylcyano-thioformamides, in two steps starting from aromatic amines.[2] Previous work had shown that the cyanothioformamides **3** may be prepared from the corresponding amines (in a two step process) and then transformed into *N*-arylimines **2**.[3,4] In comparison the route *via* **1** represents a simpler, cheaper and higher yielding method of preparing **2** and **3** for which some significant biological activity against some fungi, grasses and broad-leaved weeds was described.[4,5]



As part of our work, we increased the range of aromatics amines that condense with 4,5-dichloro-dithiazolium chloride **1** and we varied the structure of the aryl groups in imines **2** and thioformamides **3**.[6] Thus, nucleophilic neighbouring groups such as methyl ester, *o*-methoxy or nitrile were introduced into the *ortho* position of the aromatic ring in the hope of enhancing the biological activity of the products. The influence of an electron-releasing aryl substituent, such as methoxy, was also studied.

Chemistry.

Primary aromatic amines were condensed with 4,5-dichloro-1,2,3,-dithiazolium chloride **1** in dichloromethane at room temperature, followed by the addition of pyridine, to give the stable crystalline iminodithiazoles **2**.[6,7] With triphenylphosphine in moist dichloromethane at room temperature, these imines **2** gave the corresponding *N*-arylcyanothioformamides **3** in very good yields (Table 1), providing a route to these products from anilines in two mild steps.[2,6]

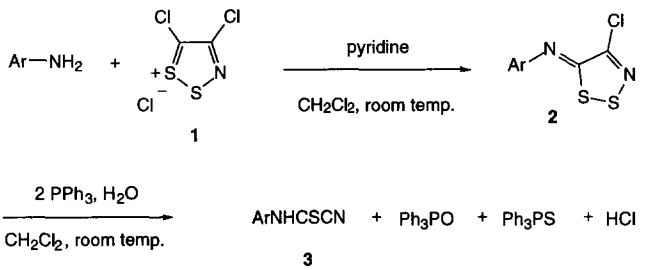
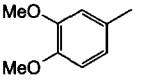
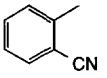
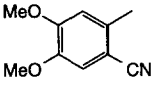
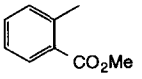
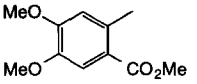
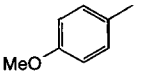
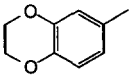
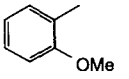
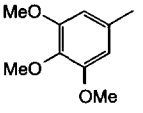


Table 1. Preparation of the *N*-arylimines **2** and *N*-arylcyanothioformamides **3**.[8]

Product	Ar moiety	Yield of products (%) 2	3	Product	Ar moiety	Yield of products (%) 2	3
a		60[a]	–	f		47	98
b		78	-[b]	g		76	76
c		71	51	h		78	93
d		71[c]	81[d]	i		60	80
e		73	–	j		86	–

[a] Spectral data in accordance with values described in ref. 3 and 4; [b] Unstable compound; [c] Spectral data in accordance with values described in ref. 3; [d] Product already available by treatment of **2d** with *m*-CPBA as described in ref. 6.

Biological evaluation.

The *N*-arylimines **2** and *N*-arylcyanothioformamides **3** were tested for their *in vitro* antibacterial activity against the following bacterial strains: Gram-negative bacteria, *Escherichia Coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* Lab.coll.[9], *Proteus mirabilis* CIP 1031811, *Salmonella choleraesuis* ser. typhimurium Lab. coll.[9] and Gram-positive bacteria, *Staphylococcus aureus* ATCC 9144, *Streptococcus pyogenes* ATCC 19165, *Listeria monocytogenes* CIP 82110T, *Enterococcus faecalis* ATCC 29212.[10]

For all the *N*-arylimines **2** or *N*- cyanothioformamides **3**, the antimicrobial assays (performed by the disk diffusion method[11]) showed that the growth of the Gram-negative bacteria on solid media was not affected. All the *N* -arylcyanothioformamides **3** were also found to be inactive against Gram-positive bacteria. In contrast, the *N*-arylimines **2** showed significant antibacterial activity against the Gram-positive bacteria (Table 2). The minimum inhibitory concentrations (MICs) were determined by the broth dilution method[12,13] (Table 3).

Table 2. Antibacterial activities of compounds **2** by the agar disk diffusion method[11]

Compound (30 μg)	Zone diameter limit (mm)[a]			
	S.aureus	*E.faecalis*	*S.pyogenes*	*L.monocytogenes*
2a	29	19	26	27
2b	11	11	15	11
2c	18	16	18	17
2d	17	14	15	15
2e	20	17	22	21
2f	13	13	16	12
2g	10	10	13	10
2h	17	14	14	17
2i	12	10	11	10
2j	13	10	16	17

[a]. The average diameter of clear zone (mm), measured in triplicate.

Table 3. Minimum Inhibitory Concentration (μg/ml)[a]

Compound	Bacteria tested			
	S.aureus	*E.faecalis*	*S.pyogenes*	*L.monocytogenes*
2a	16	16	32	16
2b	32	32	32	32
2c	32	32	32	32
2d	32	32	32	32
2e	32	16	32	16
2f	32	32	32	32
2g	32	32	32	32
2h	32	32	32	32
2i	32	32	32	32
2j	32	32	32	32

[a]. Measured in triplicate.

Several experiments showed that the cyanothioformamide functionality did not confer any activity to the molecule. It is evident that the 1,2,3-dithiazole ring is adequate for significant inhibitory activity against Gram-positive microorganisms. The unsubstituted aromatic compound **2a** and its *o*-methoxy derivative **2e** appear to be the most active of the series tested. Detailed studies determining the mechanism of action of these compounds on the bacteria will be published later.

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6. Besson, T.; Rees, C. W. *J. Chem. Soc., Perkin Trans 1* **1995**, 1659.
7. Appel, R.; Janssen, H.; Siray, M; Knoch, F. *Chem. Ber.* **1985**, *118*, 1632.
8. All compounds were prepared according methods previously described in ref. 2,3 and 6 and were fully characterised by spectroscopy and elemental analysis.
   N-*(4-Chloro-5*H*-1,2,3-dithiazol-5-ylidene)aniline derivatives 2; general procedure.* To a solution of the substituted aniline in dichloromethane was added 4,5-dichloro-1,2,3-dithiazolium chloride **1** (1 equiv.). The mixture was stirred at room temperature for 2 h after which pyridine (2 equiv.) was added to give a red solution. This was stirred for a further 2 h, filtered and the product isolated by flash column chromatography with light petroleum-dichloromethane as the eluent.
   N-*(Cyanothioformyl)anilines derivatives 3; general procedure.* A solution of the *N*-arylimine **2** and triphenylphosphine in undried dichloromethane was stirred at room temperature. The reaction was followed by TLC and when complete the product was purified by flash chromatography (eluent: light petroleum-dichloromethane) to give the title compounds.
9. Lab.coll.: Laboratory collection.
10. All the bacteria were grown on agar plates (37°C, 24 h), except *S. pyogenes* which was grown on 5% sheep blood agar plates.
11. Barry, A.L.; Thornsberry, C. In *Susceptibility tests: Diffusion test procedures*; Balows, A.; Hausler, W.J.; Herrmann, K.L.Jr.;.Isenberg, H.D.; Shadomy, H.J. (Ed.): Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.,1991; p.1117-1125.
12. MICs were determinated by the macrodilution broth method.[13] The tested compounds were first dissolved in DMF. The concentration of DMF was always 1% in Mueller-Hinton broth, that did not affect the growth of any of the microorganisms employed.
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