Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 5, pp. 529-532, 1996

0960-894X/96 $15.00 + 0.00

S0960-894X(96)00062-5

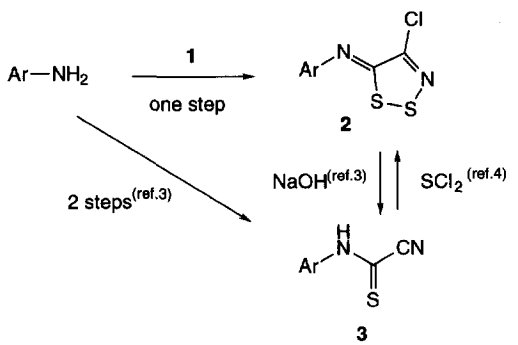
Antibacterial Evaluation of Novel *N*-Arylimino-1,2,3-dithiazoles and *N*-Arylcyanothioformamides.

Gilles Cottenceau[a], Thierry Besson*[a1], Valérie Gautier[a], Charles W. Rees[b] and Anne-Marie Pons[a]

a) Laboratoire de Génie Protéique et Cellulaire, Pôle des Sciences et Technologies,
Université de La Rochelle, Avenue Marillac, 17042 La Rochelle cedex 1, France
b) Department of Chemistry, Imperial College of Science, Technology and Medicine, London SW7 2AY, UK

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-dichlorodithiazolium 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]

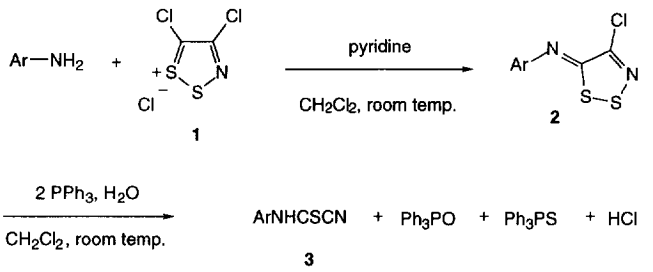
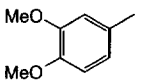
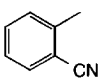
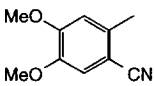
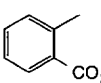
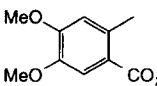
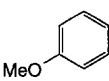
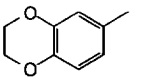
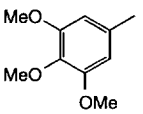


Table 1. Preparation of the *N*-arylimines **2** and *N*-arylcyanothioformamides **3**.[8]

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.

Acknowledgements.

We thank the *Comité de Charente Maritime de La Ligue Nationale contre le Cancer* for financial support, the Wolfson Foundation for establishing the Wolfson Centre for Organic Chemistry in Medical Science at Imperial College, and Pr M. D. Legoy for generous support.

REFERENCES AND NOTES.

1. Groupe de Chimie Organique et Biocatalyse, e-mail: tbesson@bio.univ-lr.fr
2. Besson, T.; Emayan, K.; Rees, C. W. *J. Chem. Soc., Perkin Trans. 1* **1995**, 2097; Besson, T.; Emayan, K.; Rees, C. W. *J. Chem. Soc., Chem. Commun.* **1995**, 1419.
3. English, R.F., Ph. D. Thesis, University of London, 1989. Rees, C. W. *J. Heterocycl. Chem.* **1992**, *29*, 639.
4. Moore, J. E. *US Pat.*, 4 059 590/1977 (*Chem. Abstr.* **1978**, *88*, 50874).
5. Mayer, R.; Förster, E.; Matauschek, B. *D. German Pat.*, DD 212 387/1984 (*Chem. Abstr.* **1985**, *102*, 113064).
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.
13. Sahm,D.F.and Washington,J.A. In *Antibacterial susceptibility tests: Dilution methods*; 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.1105-1116.

(*Received in Belgium* 28 *November* 1995; *accepted* 25 *January* 1996)