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Antimicrobial Evaluation of 3,1-Benzoxazin-4-ones, 3,1-Benzothiazin-4-ones, 4-Alkoxyquinazolin-2-carbonitriles and *N*-Arylimino-1,2,3-dithiazoles

Chemistry
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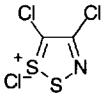
Abstract: Novel 3,1-benzoxazinones, 3,1-benzothiazinones and 4-alkoxyquinazolines have been synthesized *via* *N*-aryl-1,2,3-dithiazoles derivatives. The antibacterial and antifungal activity of these compounds were measured; the dithiazoles are significantly active against fungi. Copyright © 1996 Elsevier Science Ltd

We recently described the synthesis and antibacterial activity of novel *N*-arylimino-1,2,3-dithiazoles **2** and *N*-arylcyanothioformamides **3** and we reported that the dithiazoles derivatives are significantly active against Gram-positive bacteria.[1] As part of our work, we increased the range of aromatics amines that condense with the salt **1** and we varied the structure of the aryl groups in imines **2**.[1,4] Thus, neighbouring groups such as carboxylic acid and nitrile were introduced into the *ortho* position of the aromatic ring of the imines **2** allowing the formation of novel 3,1-benzoxazin-4-ones **4**, 3,1-benzothiazin-4-ones **5**[4] and 4-alkoxyquinazolines **6** and **7**[5] derivatives of which continue to be of interest because of their diverse biological activity (*e. g.* potent alternate inhibitors of human leucocyte elastase, C1r serine protease or porcine cyclic GMP phosphodiesterase).[6]



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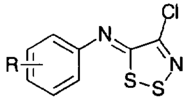

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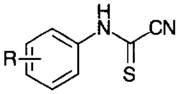

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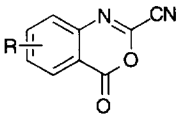

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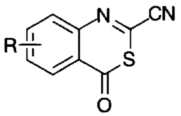

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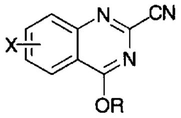

6 (X = 6,7-diOMe)
7 (X = H)

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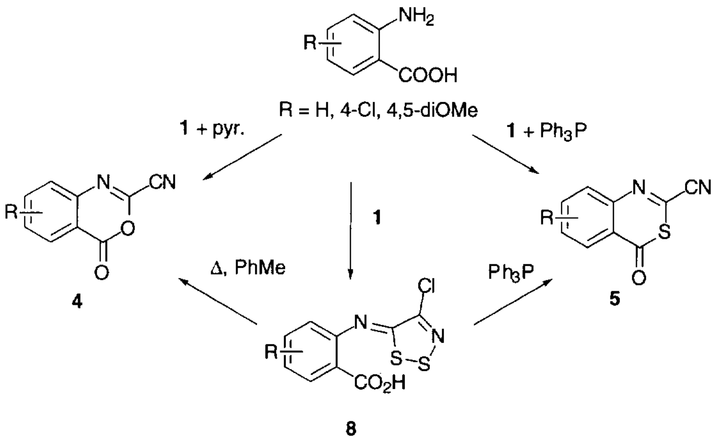
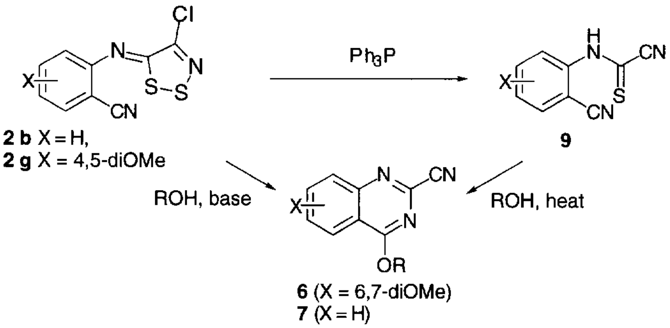
We have now measured the antibacterial and antifungal activity of compounds **4**, **5**, **6** and **7**, obtained by chemical transformation of the active imines **2**.

Chemistry

Primary aromatic amines can be 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** (Table 1).[7] Anthranilic acid and its benzo-substituted derivatives, such as 4-chloro and 4,5-dimethoxy-anthranilic acid, behaved differently to all the other anilines investigated.[4] With dithiazolium chloride **1**, as above, it did not give the analogous imines **2** but rather 2-cyano-3,1-benzoxazin-4-ones derivatives **4**, and with triphenylphoshine it gave 2-cyano-3,1-benzothiazin-4-ones **5**. However, when an excess of anthranilic acid (4 equiv.) was treated with dithiazolium chloride **1** without addition of the usual base (pyridine), the delicate iminoderivatives **8** of the free carboxylic acids could be isolated in good yield (Table 1). Heating these compounds in boiling toluene afforded benzoxazinones **4** in good yield, and treatment with triphenylphosphine (2 equiv.) in dichloromethane readily gave the corresponding benzothiazinones **5**.[4]



Heating the *o*-cyanoarylimines **2b** and **2g**, from anthranilonitrile derivatives and the salt **1**, with an alcohol and a base gave the 4-alkoxy-2-cyanoquinazolines **6** in good yields (63-82%). However, the yields of products **6b**, **7a** and **7b** were much improved, in a shorter reaction time, by microwave irradiation of the reaction mixture (Table 2).



Alternatively imines **2b** and **2g** can be converted into the more reactive cyanothioformamides **9** by treatment with triphenylphosphine (2 equiv.) in moist dichloromethane. A brief heating of **9** in an alcohol gave the corresponding 4-alkoxyquinazolines **6** and **7** in high yields (76-97%).[5]

Table 1. Preparation of the *N*-arylimines **2** and **8**.

Product	R	Yield (%)	Ref.	Product	R	Yield (%)	Ref.
2a	H	60	1,2	**2h**	2-CO_2Me, 4,5-diOMe	78	1,4
2b	2-CN	78	1,5	**2i**	4,5-OCH_2CH_2O	60	1
2c	2-CO_2Me	71	1,3	**2j**	3,4,5-triOMe	86	1
2d	4-OMe	71	1	**8a**	2-CO_2H	60	4
2e	2-OMe	73	1,2	**8b**	2-CO_2H, 4,5-diOMe	53	4
2f	3,4-diOMe	47	1,4	**8c**	2-CO_2H, 5-Cl	85	4
2g	2-CN, 4,5-diOMe	76	1,4,5				

Table 2. Preparation of the 3,1-benzoxazinones **4**, benzothiazinones **5** and quinazolines **6** and **7**.[8]

Product	R	Yield of product	m.p. (°C)	Solvent[a]	Formula
4a	H	99[b]	126	P.E./DCM	$C_9H_4N_2O_2$
4b	7-Cl	30[b]	101	P.E./DCM	$C_9H_3ClN_2O_2$
4c	6,7-diOMe	95[b]	175	P.E./DCM	$C_{11}H_8N_2O_4$
5a	H	100[b]	122	P.E./DCM	$C_9H_4N_2OS$
5b	7-Cl	42[b]	108	P.E./DCM	$C_9H_3ClN_2OS$
5c	6,7-diOMe	49[b]	194	P.E./DCM	$C_{11}H_8N_2O_3S$
6a	Me	77[c]	238	MeOH	$C_{12}H_{11}N_3O_3$
6b	Et	80[d]	228	EtOH	$C_{13}H_{13}N_3O_3$
6c	Pr^i	63[c]	206	iPrOH	$C_{14}H_{15}N_3O_3$
6d	*n*-Bu	82[c]	174	*n*-BuOH	$C_{15}H_{17}N_3O_3$
6e	SEt for OR	24[e]	194	P.E./DCM	$C_{13}H_{13}N_3O_2S$
7a	Me	41[f]	130	-	$C_{10}H_7N_3O$
7b	Et	80[f]	140	P.E./DCM	$C_{11}H_9N_3O$

[a] Solvent of recrystallization (P.E.: light petroleum, b.p. 40-60°C; DCM : dichloromethane). [b] From imines **8**. [c] Conventional heating from imine **2g**. [d] Microwave experiment from imine **2g**. [e] Prepared *via* the cyanothioformamide **9** (R = 4,5-diOMe). [f] Microwave experiment from the imine **2b**.

Biological evaluation

Antibacterial activity.

As previously described for the imines **2**[1] the antibacterial *in vitro* activity of derivatives **4**, **5**, **6** and **7** were evaluated against Gram-negative bacteria (*Escherichia Coli* ATCC[9] 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* Lab. coll.,[9] *Proteus mirabilis* CIP[9] 103181l, *Salmonella choleraesuis* ser. Typhimurium Lab. coll.) and Gram-positive bacteria (*Staphylococcus aureus* ATCC 9144, *Streptococcus pyogenes* ATCC 19165, *Listeria monocytogenes* CIP 82110T, *Enterococcus faecalis* ATCC

29212).[10] The antimicrobial assays were performed by the disk diffusion method.[11,12] None of the tested compounds **4**, **5**, **6** and **7** had any effect on the growth of both Gram-positive and Gram-negative bacteria.

Antifungal activity.

The antifungal activity of compounds **2**, **4**, **5** and **7** was studied. The experiments were performed with the following pathogenic strains : *Candida albicans* ATCC[9] 10231, *Candida glabrata* DSM[9] 6425, *Candida tropicalis* DSM 1346, *Issatchenkia orientalis* DSM 6128, *Cryptococcus neoformans* DSM 70219.[13] A preliminary antifungal testing was worked out using the agar diffusion method.[14,15] No activity was found except for the *N*-aryliminodithiazoles **2** These results are similar to those recently reported for another sulfur heterocycle, the dithiins; these have a lethal action *in vitro* against several pathogenic fungi (*e. g. Candida albicans, Cryptococcus neoformans, Aspergillus fumigatus*).[16] The minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC_{99}, defined as the lowest concentration of drug that killed 99% of the inoculum) were then determinated using the broth dilution method.[14,15] The results are summarized in Table 3. Amphotericin B (AMB), fluconazole (FLU) and flucytosine (5-FC) were used as reference products for inhibitory activity against fungi.

Table 3. Fungicidal activities of *N*-aryliminodithiazoles, AMB, FLU and 5-FC for yeast strains.

	Fungi tested[a]														
Compound	*C. albicans*			*C. glabrata*			*C. tropicalis*			*I. orientalis*			*C. neoformans*		
	MIC	*MFC_{99}*	*R*[b]	*MIC*	*MFC_{99}*	*R*[b]	*MIC*	*MFC_{99}*	*R*[b]	*MIC*	*MFC_{99}*	*R*[b]	*MIC*	*MFC_{99}*	*R*[b]
2a	16	16	1	16	16	1	16	16	1	16	16	1	8	8	1
2b	16	16	1	16	16	1	16	16	1	16	16	1	16	16	1
2c	16	16	1	16	32	2	16	16	1	16	32	2	32	32	1
2d	16	16	1	16	16	1	16	16	1	16	16	1	8	8	1
2e	16	16	1	16	16	1	16	16	1	16	16	1	8	16	2
2f	16	16	1	16	16	1	16	32	2	16	32	2	16	32	2
2g	32	32	1	32	48	1,5	48	>48	-	32	48	1,5	32	32	1
2h	32	48	1,5	16	48	3	48	>48	-	32	48	1,5	32	48	1,5
2i	32	48	1,5	16	48	3	48	>48	-	48	>48	-	16	16	1
2j	16	16	1	16	16	1	16	32	2	16	32	2	16	32	2
AMP	0,25	0,5	2	1	2	2	1	2	2	0,5	1	2	0,13	0,5	3,8
FLU	2	>128	>64	4	>128	>32	4	>128	>32	16	64	4	4	>128	>32
5-FC	0,13	4	30	0,05	0,25	5	0,1	0,25	5	16	64	4	2	>128	>64

[a] Measured in triplicate; MIC and MFC_{99} in µg/ml. [b] R= MFC_{99}/MIC ratio.

All the derivatives **2** showed significant antifungal activity against the yeasts tested. However compounds **2a** (R = H) and **2e** (R = 2-OMe) are always the most active, as we observed with the antibacterial results. For the five yeasts strains tested, MFC_{99} of the products **2** were no more than two or threefold greater than the MIC showing a good and significant fungicidal activity. These results are similar to those generally observed with amphotericin and flucytosine. In contrast, the triazole fluconazole did not kill most yeast strains when it was used at concentrations close to the MICs for the strains.

Although fluconazole, amphotericin B and flucytosine have a higher activity against pathogenic yeasts than the *N*-aryliminodithiazoles **2** tested, they have some limitations (*e.g.* nephrotoxicity of amphotericin, lesser for the new forms, limits its utilization as fungicidal agent; fluconazole has only fungistatic activity and resistance to flucytosine is commonly found). Based on their fungicidal activity, our iminodithiazoles **2** may represent interesting leeds for further study.

As we suggested in our previous work on the antibacterial activity on Gram-positive microorganisms,[1] it seems likely that the 1,2,3-dithiazole ring is implicated in inhibitory activity against fungi.

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8. All compounds were fully characterised by spectroscopy and elemental analysis. 4-Oxo-*4H*-3,1-benzoxazine-2-carbonitrile derivatives **4** and 4-oxo-*4H*-benzo-3,1-thiazine-2-carbonitrile derivatives **5** were prepared from *N*-(4-chloro-*5H*-1,2,3-dithiazol-5-ylidene)-anthranilic acid according methods previously described in ref. 2-6. *4-Substituted-quinazoline-2-carbonitriles (e.g. 6 and 7). General procedure from imines 2.* (a) Conventional heating: a stirred mixture of nitriles **2b** and **2g** (1 mmol) and sodium hydride (NaH, 1 mmol) in the alcohol (5 ml) was heated at reflux for 40 h. The product was purified by column chromatography. (b) Microwave experiments: the reaction mixture was placed in microwave oven (Synthewave S402 Prolabo® microwave reactor (monomode system) which has a quartz reactor, variable speed rotation, visual control, irradiation monitored by PC computer, infrared measurement and continuous feedback temperature control by PC) in an open vessel. The irradiation was programmed for 2 h with a delay of 10-15 seconds to obtain reflux. The product was purified as described above.
9. Lab.coll.: Laboratory collection; ATCC: American Type Culture Collection; DSM: Deutsche Sammlung von Mikroorganismen,und Zellkulturen GmbH; CIP: Collection Institut Pasteur.
10. All the bacteria were grown on nutrient agar plates (35 °C, 24 h), except *S. pyogenes* which was grown on 5% sheep blood agar plates.
11. The tested compounds were first dissolved in DMF. The concentration of DMF was always 1% in the Mueller-Hinton broth, which did not affect the growth of any of the bacteria employed.
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13. Yeasts were grown on Sabouraud dextrose agar plates (37° C, 24 h).
14. The tested compounds were first dissolved in DMF. The concentration of DMF was always 1% in RPMI 1640 (with L-glutamine) buffered to pH 7.0 with 0.165 M MOPS buffer which did not affect the growth of any of the yeasts employed.
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