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Short communication

Antimicrobial activity of novel *N*-quinolinyl and *N*-naphthylimino-1,2,3-dithiazoles

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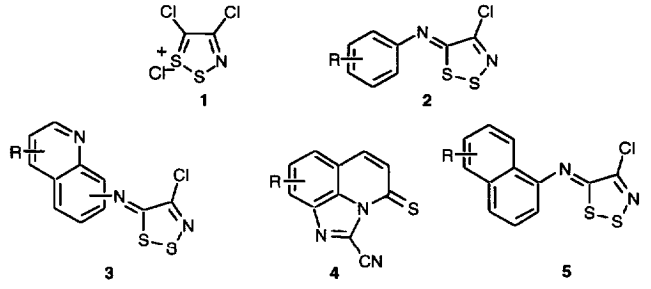
Abstract – Novel *N*-arylimino-1,2,3-dithiazole derivatives of aminoquinolines and aminonaphthalenes have been synthesised. The antibacterial and antifungal activities of these compounds were measured; the new dithiazoles are significantly active against fungi, but no enhancement of biological activity was detected by introduction of the more complex aromatic moiety. © Elsevier, Paris

imino-1,2,3-dithiazoles / quinolines / antimicrobial activity

1. Introduction

It is now well known that reaction of 4,5-dichloro-1,2,3-dithiazolium chloride **1** [1] with primary aromatic amines in dichloromethane at room temperature allows access to stable *N*-arylimino-4-chloro-5*H*-1,2,3-dithiazoles **2** [1–4]. These compounds are important key synthetic intermediates and have shown interesting biological activity (e.g. as fungicides, acaricides and herbicides) [5]. We recently reported the antimicrobial evaluation of such imines prepared from various anilines [6]. Studying the chemistry of the salt **1** and its derivatives, we synthesised the relatively rare 4*H*-imidazo[5,4,1-*ij*]quinolines **4** in two steps from 8-aminoquinolines via pyrolysis of the intermediate *N*-(8-quinolinyl)-iminodithiazoles **3** [7]. These novel imines represent an extension of the aromatic heterocyclic moiety compared to the compounds obtained by condensation of anilines with the dithiazolium salt **1** (*scheme 1*). Expecting an enhancement of the antimicrobial potential of such quinolinyliminodithiazoles **3** we have measured their antibacterial and antifungal activity. Two naphthalene derivatives **5** were also tested.



Scheme 1.

2. Chemistry

Increasing the range of aromatic amines that condense with the salt **1** we varied the position of the amino group on the quinoline ring. Standard methods were applied for the preparation of *N*-arylimines **3**; the starting aminoquinolines were condensed with 4,5-dichloro-1,2,3-dithiazole chloride **1** (1 equiv) in dichloromethane at low temperature (–20 °C) followed by addition of pyridine (2 equiv) [1–4]. The iminodithiazoles **3** were purified by column chromatography (silica gel) in good yields. Two naphthalene derivatives **5** were also synthesised (*scheme 2*, *table I*).

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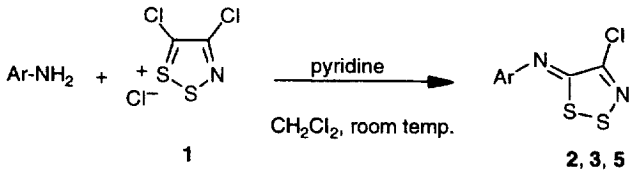


Scheme 2.

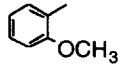
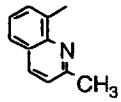
3. Biological results and discussion

Following procedures previously described for imines **2** [6], the in vitro antibacterial and the antifungal activities of the quinolinyl **3** and naphthyliminodithiazoles **5** were evaluated and compared with the unsubtituted imino-1,2,3-dithiazole **2a** and its *o*-methoxy derivative **2b** against pathogenic or opportunistic pathogenic microbial strains from Gram-positive or negative bacteria and yeasts.

3.1. Antibacterial activity

The antibacterial in vitro activity was first measured by the disk-diffusion method. None of the tested compounds had any effect on the growth of the Gram-negative bacteria. In contrast, all the Gram-positive bacteria were significantly inhibited (*table II*). The minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC_{99}, defined as the lowest concentration of product that killed 99% of the inoculum) were determined by the macrodilution-broth method (*table III*).

Table I. Synthesis of quinolyliminodithiazoles **3** and naphthyliminodithiazoles **5**.

Starting amine (Ar)	Product	Yield (%)	M.p. (°C)	Formula
	**2a**	60[a,b]	oil	$C_8H_5ClN_2S_2$
	**2b**	73[a,b]	76	$C_9H_7ClN_2OS_2$
	**3a**	65	oil	$C_{11}H_6ClN_3S_2$
	**3b**	80	202	$C_{11}H_6ClN_3S_2$
	**3c**	75	158	$C_{11}H_6ClN_3S_2$
	**3d**	53	170 (dec.)	$C_{12}H_8ClN_3S_2$
	**5a**	28	100	$C_{12}H_7ClN_2S_2$
	**5b**	50	188	$C_{12}H_7ClN_2OS_2$

[a]Spectral data in accordance with values given in [1]; [b]reaction carried out in dichloromethane at room temperature.

Table II. Antibacterial activity by the agar diffusion method. Zone diameter limit (mm)[a].

Compound (30 µg)	Bacteria tested			
	S. aureus	*E. faecalis*	*S. pyogenes*	*L. monocytogenes*
2a	28	19	27	26
2b	19	17	21	20
3a	14	14.5	14	14
3b	8	–	9	7
3c	13	12	11	11
3d	13	13	13.5	8
5a	11	9.5	12	10
5b	11.5	12	13	12

[a]Average diameter of the clear zone (mm), measured in triplicate.

Table III. Bactericidal activities, MIC and MBC_{99} (μg/mL)[a].

Compound	Bacteria tested											
	S. aureus			*E. faecalis*			*S. pyogenes*			*L. monocytogenes*		
	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	32	32	1	16	16	1
2b	32	32	1	16	16	1	32	32	1	16	16	1
3a	32	> 48	–	48	> 48	–	32	48	1.5	32	> 48	–
3b	32	> 48	–	32	> 48	–	32	48	1.5	32	> 48	–
3c	32	> 48	–	32	> 48	–	32	> 48	–	32	> 48	–
3d	32	> 48	–	48	> 48	–	32	32	1	32	> 48	–
5a	16	32	2	16	> 48	–	16	32	2	16	> 48	–
5b	16	32	2	16	32	2	16	16	1	16	32	2

[a]Measured in triplicate; [b]R = MBC_{99}/MIC ratio.

Table IV. Fungicidal activities, MIC and MFC_{99} (μg/mL)[a].

Compound	Fungi tested											
	C. albicans			*C. glabrata*			*C. tropicalis*			*I. orientalis*		
	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
2b	16	16	1	16	16	1	16	16	1	16	16	1
3a	16	16	1	16	48	3	16	48	3	16	48	3
3b	32	32	1	32	48	1.5	48	> 48	–	48	> 48	–
3c	16	16	1	16	32	2	32	48	1.5	32	32	1
3d	32	32	1	32	48	1.5	48	48	1	48	48	1
5a	16	16	1	32	32	1	32	32	1	32	32	1
5b	16	16	1	32	48	1.5	48	48	1	48	48	1
AMP	0.25	0.5	2	1	2	2	1	2	2	0.5	1	2
FLU	2	> 128	> 64	4	> 128	> 32	4	> 128	> 32	16	64	4
5-FC	0.13	4	30	0.05	0.25	5	0.1	0.25	5	16	64	4

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

3.2. *Antifungal activity*

The antifungal activity of products **3** and **5** was initially tested using the agar-diffusion method. All the tested products showed inhibitory activity. These results are similar to those that we have previously obtained with similar imines [6]. The fungicidal activity was then evaluated by the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC_{99}) using the macrodilution broth method (*table IV*). Amphotericin B (AMB), fluconazole (FLU) and flucytosine (5-FC) were used as reference products for inhibitory activity against fungi.

Compounds **2** and **5** showed significant bactericidal and fungicidal activity against tested strains ($R < 3$). However, compounds **2a** (R = H) and **2b** (R = 2-OMe) are always the most active as we observed previously with other derivatives [6]. For the four fungi tested, MFC_{99} of products **2** and **5** were no more than two times 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 has only fungistatic activity. It seemed to be evident now that the aromatic portion of the molecule does not interfere with the antimicrobial activity which probably depends on the 1,2,3-dithiazole ring acting as a potent inhibitor of some enzymes like serine proteases. Further investigations are under way to assess the role of the dithiazole ring and to elucidate the mechanism of inhibition on microorganisms.

4. Experimental protocols

4.1. *Chemistry*

Melting points were determined using a Kofler banc and are uncorrected. IR spectra were recorded on a Perkin-Elmer Paragon 1000PC instrument. ^{1}H- and ^{13}C-NMR were recorded on a JEOL JNM LA400 (400 MHz) spectrometer (Laboratoire Commun d'Analyse, Université de La Rochelle); chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS), which was used as internal standard. Mass spectra were recorded on a Varian MAT311 in the Centre de Mesure Physiques de L'Ouest (C.R.M.P.O.), Université de Rennes. Chromatography was carried out on silica gel 60 at medium pressure and the sample mixtures were applied to the column preadsorbed onto silica. Light petroleum refers to the fraction b.p. 40–60 °C. Further solvents were used without purification. Thin-layer chromatography was performed on Merck Kieselgel 60 F_{254} aluminium baked plates. Compounds **2** were prepared following procedures previously described in [1]. Spectral data are consistent with assigned structures.

4.1.1. *Imino-1,2,3-dithiazoles from aromatic amines: general procedure*

Under an inert atmosphere (argon), dithiazolium salt **1** (0.208 g, 1 mmol) was added to a solution of aromatic amine (1 mmol) in dichloromethane (5 mL). The mixture was cooled (–20 °C) and pyridine (2 mmol) was added. The mixture was stirred until all of the amine had been used up (tlc control). The mixture was warmed to room temperature and the reaction mixture filtered through acidic alumina and poured into ice water to separate the organic layer. The aqueous layer was washed with dichloromethane; the combined organics layers were washed with $NaHCO_3$ (saturated solution) and dried over magnesium sulfate. The crude product was purified by column chromatography using dichloromethane/ethylacetate as the eluent.

4.1.2. *8-N-(4-Chloro-5H-1,2,3-dithiazol-5-ylidene)aminoquinoline* ***3a***

Treatment of 8-aminoquinoline (1 g, 6.9 mmol) gave 1.25 g of product **3a** as a yellow brown oil; yield 65%; IR (film) ν 1600, 1496, 1160 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 8.91 (dd, 1H, C2-H, $J_{2,3}$ = 4.2 Hz, $J_{2,4}$ = 1.7 Hz), 8.21 (dd, 1H, C4-H, $J_{2,4}$ = 1.7 Hz, $J_{3,4}$ = 8.4 Hz), 7.71 (dd, 1H, C5-H, $J_{5,7}$ = 1.4 Hz, $J_{5,6}$ = 8.1 Hz), 7.59 (t, 1H, C6-H, $J_{5,6}$ = 8.1 Hz, $J_{6,7}$ = 7.4 Hz), 7.49–7.43 (m, 2H, C3-H, C7-H). MS (EI) *m/z* 279 (M^+), 244 (M^+ – Cl), 211 (M^+ – HCl – S), 186 (M^+ – ClCNS, 85%). HRMS: calc. for $C_{11}H_6ClN_3S_2$: 278.96917, found 278.9698.

4.1.3. *5-N-(4-Chloro-5H-1,2,3-dithiazol-5-ylidene)aminoquinoline* ***3b***

Treatment of 5-aminoquinoline (1 g, 6.9 mmol) gave 1.55 g of product **3b** as orange needles; yield 80%; m.p. 202 °C (from ethanol). IR (KBr): ν 1557, 1503, 1464, 1391, 1135 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.51–7.45 (m, 2H, C3-H, C8-H), 7.78 (t, 1H, C7-H, $J_{5,7}$ = 8.3 Hz, $J_{6,7}$ = 7.4 Hz), 8.04 (d, 1H, C6-H, $J_{6,7}$ = 8.3 Hz), 8.64 (dd, 1H, C4-H, $J_{2,4}$ = 1.4 Hz, $J_{2,3}$ = 8.4 Hz), 8.99 (dd, 1H, C2-H, $J_{2,3}$ = 4.0 Hz, $J_{2,4}$ = 1.4 Hz); MS (EI) *m/z* 279 (M^+), 244 (M^+ – Cl), 211 (M^+ – HCl – S), 186 (M^+ – ClCNS). HRMS: calc. for $C_{11}H_6ClN_3S_2$: 278.96917, found 278.9698.

4.1.4. *6-N-(4-Chloro-5H-1,2,3-dithiazol-5-ylidene)aminoquinoline* ***3c***

Treatment of 6-aminoquinoline (1 g, 6.9 mmol) gave 1.45 g of product **3c** as yellow needles; yield 75%; m.p. 158 °C; IR (KBr) ν 1626, 1557, 1539, 1412, 1155 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.44 (dd, 1H, C3-H, $J_{2,3}$ = 4.4 Hz, $J_{3,4}$ = 8.3 Hz), 7.64 (m, 2H, 1H arom., C4-H), 8.22 (m, 2H arom.), 8.92 (dd, 1H, C2-H, $J_{2,3}$ = 4.4 Hz, $J_{2,4}$ = 1.4 Hz); MS (EI) *m/z* 279 (M^+), 244 (M^+ – Cl), 211 (M^+ – HCl – S), 186 (M^+ – ClCNS, 85%). HRMS: calc. for $C_{11}H_6ClN_3S_2$: 278.96917, found 278.9698.

4.1.5. *2-Methyl-8-N-(4-chloro-5H-1,2,3-dithiazol-5-ylidene)-aminoquinoline* ***3d***

Treatment of 2-methyl-8-aminoquinoline (1 g, 6.3 mmol) gave 0.98 g of product **3d** as brown needles; yield 53%; m.p. 170 °C (dec.); IR (KBr) ν 1605, 1565, 1465, 1155 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 8.07 (d, 1H, C4-H, $J_{3,4}$ = 8.8 Hz), 7.65 (dd, 1H, C7-H, $J_{6,7}$ = 7.8 Hz, $J_{5,7}$ = 1.2 Hz), 7.53–7.45 (m, 2H arom., C5-H, C6-H), 7.32 (d, 1H, C3-H, $J_{3,4}$ = 8.8 Hz), 2.73 (t, 3H, CH_3). MS (EI) *m/z* 293 (M^+), 258 (M^+ – Cl), 225 (M^+ – HCl – S, 30%), 200 (M^+ – ClCNS, 100%), 168 (M^+ – $ClCNS_2$, 60%), 142 (M^+ – $ClCNS_2N$, 70%). HRMS: calc. for $C_{12}H_8ClN_3S_2$: 292.98467, found 278.9849.

4.1.6. *1-N-(4-Chloro-5H-1,2,3-dithiazol-5-ylidene)naphtalene* ***5a***

Treatment of 1-aminonaphtalene (1 g, 6.93 mmol) gave 0.54 g of product **5a** as red needles; yield 28%; m.p. 100 °C; IR (KBr) ν 3034, 2920, 1596, 1523, 1435, 1340, 264, 1139 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.36 (d, 1H arom., J = 6.8 Hz);

7.60–7.52 (m, 3H arom.), 7.77 (d, 1H arom., J = 4.1 Hz), 7.87 (dd, 1H arom., J = 4.1 Hz and J = 6.3 Hz), 8.23 (d, 1H arom., J = 6.8 Hz); MS (EI) *m/z* 278 (M^+, 65), 185 (M^+ – ClCNS, 35%), 153 (M^+ – $ClCNS_2$, 100%). HRMS: calc. for $C_{12}H_7ClN_3S_2$: 277.97384, found 277.97392.

4.1.7. 5-Hydroxy-1-N-(4-chloro-5H-1,2,3-dithiazol-5-ylidene)-naphtalene ***5b***

Treatment of 1-amino-5-hydroxy naphtalene (1 g, 6.28 mmol) gave 0.96 g of product **5b** as orange needles; yield 52%; m.p. 188 °C; IR (KBr) ν 3208, 1582, 1512, 1403, 1275, 1262 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 5.36 (s, 1H, OH), 6.93 (d, 1H arom., J = 0.6 Hz and J = 7.1 Hz), 7.40–7.35 (m, 2H arom.), 7.55 (dd, 1H arom., J = 7.4 Hz and J = 8.3 Hz), 7.79 (d, 1H arom., J = 8.4 Hz), 8.14 (d, 1H arom., J = 8.4 Hz); MS (EI) *m/z* 294 (M^+), 259 (M^+ – Cl), 217 (M^+ – Cl – CO, 8%), 226 (M^+ – HClSN, 8%), 217 (M^+ – ClCNS, 100%), 169 (M^+ – $ClCNS_2$, 50%). HRMS: calc. for $C_{12}H_7ClN_3OS_2$: 293.96883, found 293.9689.

4.2. Biological methods

4.2.1. Strains and media

All microbiological products were purchased from Biokar. All common chemicals were of analytical grade from Sigma. The microrganisms used in this study were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27863, *Staphylococcus aureus* ATCC 9144, *Streptococcus pyogenes* ATCC 19165, *Enterococcus faecalis* ATCC 29212, *Candida albicans* ATCC 10231 (American Type Culture Collection); *Candida glabrata* DSM 6425, *Candida tropicalis* DSM 1346, *Issatchenkia orientalis* DSM 6128 (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH); *Proteus mirabilis* CIP 1031811, *Listeria monocytogenes* CIP 82110T (Collection Institut Pasteur); *Klebsiella pneumoniae*, *Salmonella choleraesuis* ser typhimurium were from the laboratory collection. All the bacteria were grown on nutritive agar plates (37 °C, 24 h) except *S. pyogenes* which was grown on 5% sheep blood agar; all the yeasts were grown on Sabouraud dextrose agar plates (37 °C, 24 h).

4.2.2. Antimicrobial antagonism

Evaluation of the antibacterial and antifungal activity by the disk-diffusion method was made according to Barry [8] for the bacteria and Shadomy [9] for the yeasts. The tested compounds were dissolved in DMF in such a way that the used quantity of solvent did not affect the growth of any of the microorganisms employed. Determination of bactericidal and fungicidal characteristics of products (MIC and MBC_{99}) were made by the macrodilution broth method according to Sahm [10] for the bacteria (the tested compounds were first dissolved in DMF and the solvent concentration was always 1% in the Muller–Hinton broth, which did not affect the growth of any of the bacteria employed) and Shadomy [9] for the yeasts (the tested compounds were first dissolved in DMF and the solvent concentration was always 1% in RPMI 1640 (with L-glutamine) buffered to pH 7 with 0.165 M MOPS buffer which did not affect the growth of any of the yeasts employed).

Acknowledgements

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[1] The salt **1** is a pale greenish yellow solid, insoluble in organic solvents. It is completely stable in a dry inert atmosphere but reacts slowly with moisture to form 4-chloro-1,2,3-dithiazol-5-one: Appel R., Janssen H., Siray M., Knoch F., Chem. Ber. 118 (1985) 1632–1643.

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