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Synthesis and antimicrobial activity of novel arylideneisothiosemicarbazones

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Abstract

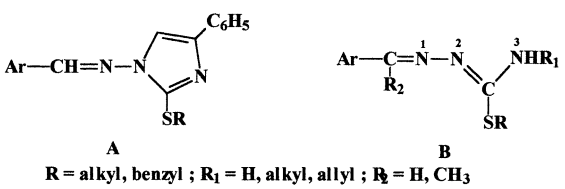
Arylidenimidazoles bearing a thioethereal function in the position 2 of the imidazole ring show good antimicrobial activity. We now report on the synthesis and the biological properties of some novel arylidenisothiosemicarbazones, structurally related to the arylideneiminoimidazoles of which they can be considerecd the linear precursors. Particular attention has been put on the influence of structural modifications on the biological activity.

Keywords: Arylideneisothiosemicarbazones; Antimicrobial; Structure–activity relationships

1. Introduction

It has been observed that some 1-amino-2,4-disubstituted imidazoles, easily obtained by reacting α-haloketones with *N*-acetylamidohydrazones, exhibit a good antimicrobial activity [1–4]. This activity is particularly evident in the arylidene derivatives shown in Scheme 1 (structure **A**).

Among such compounds, the highest antimicrobial activity was observed for the arylidenimidazoles bearing a thioethereal function in the position 2 of the imidazole ring. The introduction of alkyl or benzyl substituents in the position 2 of the imidazole ring also leads to antifungal activity. It has been reported by some of us that isothiosemicarbazones exhibit good antimicrobial activity [5]. Not much has been reported on the antimicrobial activity of these compounds in comparison with the information available for the analogous thiosemicarbazones [6–11].



Scheme 1.

In this paper we report on the synthesis and the biological properties of some new arylidenisothiosemicarbazones **B** (Scheme 1). In particular, we have investigated the influence of the following structural modifications on the biological activity.

- Length and nature of the chain linked to the sulfur atom
- Introduction of different substituents on the aromatic ring
- Substitution of the arylidene hydrogen with an alkyl group
- Substitution of the primary amine group with a secondary amine
- Substitution of the 5-nitro-furane group with other aromatic systems

In order to achieve this aim, we have synthesised and screened against different bacteria against the compounds reported in Table 1. Particular effort has been put into evaluating the structure–activity relationships of the newly synthesised compounds with respect to their antimicrobial and antifungal activity.

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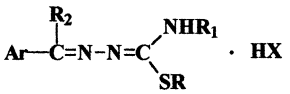
Table 1
Synthesised compounds



Comp.	Ar	R	R_1	R_2	X	m.p	Crystal solvent [a]	Yield (%)
1	2-(5-NO_2–C_4H_2O)–	*n*-C_4H_9	CH_3	H	Br	165	C	90
2	2-(5-NO_2–C_4H_2O)–	CH_2=CH–CH_2–	CH_3	H	Br	149	D	82
3	2-(5-NO_2–C_4H_2O)–	3-CH_3–C_6H_4–CH_2–	CH_3	H	Br	182	C	85
4	2-(5-NO_2–C_4H_2O)–	3-CH_3–C_6H_4–CH_2–	H	H	Br	195	C	85
5	2-(5-NO_2–C_4H_2O)–	4-Cl–C_6H_4–CH_2–	CH_3	H	Cl	191	D	88
6	2-(5-NO_2–C_4H_2O)–	CH_2=CH–CH_2–	CH_2=CH–CH_2–	H	Br	137	E	83
7	2-(5-NO_2–C_4H_2O)–	CH_3	CH_2=CH–CH_2–	H	I	159	D	82
8	2-(5-NO_2–C_4H_2O)–	CH_3	CH_3	H	I	168	D	88
9	4-Cl–C_6H_4–	CH_2=CH–CH_2–	CH_3	H	Br	169	C	86
10	4-Cl–C_6H_4–	4-Cl–C_6H_4–CH_2–	CH_3	H	Cl	193	D	81
11	4-Cl–C_6H_4–	CH_2=CH–CH_2–	CH_2=CH–CH_2–	H	Br	128	C	78
12	4-Cl–C_6H_4–	4-Cl–C_6H_4–CH_2–	CH_2=CH–CH_2–	H	Br	160	C	87
13	4-Cl–C_6H_4–	CH_3	CH_3	CH_3	I	202	F	91
14	4-Cl–C_6H_4–	CH_3	H	CH_3	I	208	F	94
15	4-Cl–C_6H_4–	4-Cl–C_6H_4–CH_2–	H	CH_3	Cl	220	D/G	88
16	4-Cl–C_6H_4–	4-Cl–C_6H_4–CH_2–	CH_3	CH_3	Cl	180	D	92
17	4-Cl–C_6H_4–	CH_2=CH–CH_2–	H	CH_3	Br	202	C	85
18	4-Cl–C_6H_4–	CH_2=CH–CH_2–	CH_3	CH_3	Br	167	E	91
19	3-Cl–C_6H_4–	*n*-C_4H_9	H	H	Br	185	C	87
20	3-Cl–C_6H_4–	CH_2=CH–CH_2–	H	H	Br	132	C	88
21	3-Cl–C_6H_4–	3-CH_3–C_6H_4–CH_2–	H	H	Br	196	C	90
22	3-Cl–C_6H_4–	4-Cl–C_6H_4–CH_2–	H	H	Cl	194	C	93
23	3-Cl–C_6H_4–	4-NO_2–C_6H_4–CH_2–	H	H	Br	195	D	92
24	3-Cl–C_6H_4–	4-Cl–C_6H_4–CH_2–	CH_3	H	Cl	196	D	92
25	3,4-Cl_2–C_6H_3–	*n*-C_4H_9	H	H	Br	190	D	82
26	3,4-Cl_2–C_6H_3–	CH_2=CH–CH_2–	H	H	Br	145	C	90
27	3,4-Cl_2–C_6H_3–	3-CH_3–C_6H_4–CH_2–	H	H	Br	206	D	88
28	3,4-Cl_2–C_6H_3–	4-Cl–C_6H_4–CH_2–	H	H	Cl	203	D	85
29	3,4-Cl_2–C_6H_3–	4-NO_2–C_6H_4–CH_2–	H	H	Br	219	D	87
30	3,4-Cl_2–C_6H_3–	CH_2=CH–CH_2–	CH_3	H	Br	180	D	87
31	3,4-Cl_2–C_6H_3–	4-Cl–C_6H_4–CH_2–	CH_3	H	Cl	207	G	77
32	3,4-Cl_2–C_6H_3–	CH_2=CH–CH_2–	CH_2=CH–CH_2–	H	Br	156	C	81
33	3,4-Cl_2–C_6H_3–	4-Cl–C_6H_4–CH_2–	CH_2=CH–CH_2–	H	Br	149	C	85
34	3,4-Cl_2–C_6H_3–	CH_3	CH_2=CH–CH_2–	H	I	175	D	78
35	3,4-Cl_2–C_6H_3–	CH_3	CH_3	H	I	194	D	80
36	3-Br–C_6H_4–	*n*-C_4H_9	H	H	Br	157	C	85
37	3-Br–C_6H_4–	CH_2=CH–CH_2–	H	H	Br	161	C	85
38	3-Br–C_6H_4–	3-CH_3–C_6H_4–CH_2–	H	H	Br	195	C	93
39	3-Br–C_6H_4–	4-Cl–C_6H_4–CH_2–	H	H	Cl	192	D	86
40	3-Br–C_6H_4–	4-NO_2–C_6H_4–CH_2–	H	H	Br	215	D	85
41	4-F–C_6H_4–	*n*-C_4H_9	H	H	Br	188	D	85
42	4-F–C_6H_4–	CH_2=CH–CH_2–	H	H	Br	168	C	89
43	4-F–C_6H_4–	3-CH_3–C_6H_4–CH_2–	H	H	Br	194	C/D	84
44	4-F–C_6H_4–	4-Cl–C_6H_4–CH_2–	H	H	Cl	202	C	78
45	4-F–C_6H_4–	4-NO_2–C_6H_4–CH_2–	H	H	Br	217	D	93
46	4-F–C_6H_4–	4-Cl–C_6H_4–CH_2–	H	H	Cl	193	F	84
47	4-F–C_6H_4–	4-Cl–C_6H_4–CH_2–	H	CH_3	Cl	223	D	90
48	4-F–C_6H_4–	4-Cl–C_6H_4–CH_2–	CH_3	CH_3	Cl	169	C	85
49	4-F–C_6H_4–	CH_3	H	CH_3	Cl	201	D	87
50	4-F–C_6H_4–	CH_2=CH–CH_2–	H	CH_3	Br	200	E	82
51	4-F–C_6H_4–	CH_2=CH–CH_2–	CH_3	CH_3	Br	186	E	78
52	4-F–C_6H_4–	CH_3	CH_3	CH_3	Br	200	E	80
53	3-F–C_6H_4–	*n*-C_4H_9	H	H	Br	189	D	81
54	3-F–C_6H_4–	CH_2=CH–CH_2–	H	H	Br	170	C	78
55	3-F–C_6H_4–	3-CH_3–C_6H_4–CH_2–	H	H	Br	180	C/D	86
56	3-F–C_6H_4–	4-Cl–C_6H_4–CH_2–	H	H	Cl	183	C	84
57	3-F–C_6H_4–	4-NO_2–C_6H_4–CH_2–	H	H	Br	190	C	88

Table 1 (Continued)

Comp.	Ar	R	R_1	R_2	X	m.p	Crystal solvent [a]	Yield (%)
58	4-CF_3–C_6H_4–	*n*-C_4H_9–	H	H	Br	201	C	88
59	4-CF_3–C_6H_4–	CH_2=CH–CH_2–	H	H	Br	168	C	87
60	4-CF_3–C_6H_4–	3-CH_3–C_6H_4–CH_2–	H	H	Br	196	C	90
61	4-CF_3–C_6H_4–	4-Cl–C_6H_4–CH_2–	H	H	Cl	168	C	80
62	4-CF_3–C_6H_4–	4-NO_2–C_6H_4–CH_2–	H	H	Br	197	D	91
63	4-CF_3–C_6H_4–	4-Cl–C_6H_4–CH_2–	CH_3	H	Cl	197	C	78
64	4-HO–C_6H_4–	CH_2=CH–CH_2–	H	H	Br	174	E	90
65	4-HO–C_6H_4–	3-CH_3–C_6H_4–CH_2–	H	H	Br	183	E	91
66	4-HO–C_6H_4–	4-Cl–C_6H_4–CH_2–	H	H	Cl	199	D	86
67	4-HO–C_6H_4–	4-NO_2–C_6H_4–CH_2–	H	H	Br	210	D	90
68	4-HO–C_6H_4–	4-Cl–C_6H_4–CH_2–	CH_3	H	Cl	208	F	85
69	4-CH_3O–C_6H_4–	*n*-C_4H_9–	H	H	Br	173	C	90
70	4-CH_3O–C_6H_4–	CH_2=CH–CH_2–	H	H	Br	152	C	85
71	4-CH_3O–C_6H_4–	3-CH_3–C_6H_4–CH_2–	H	H	Br	184	D	90
72	4-CH_3O–C_6H_4–	4-Cl–C_6H_4–CH_2–	H	H	Cl	185	C/D	97
73	4-CH_3O–C_6H_4–	4-NO_2–C_6H_4–CH_2–	H	H	Br	215	D	87
74	4-CH_3–C_6H_4–	CH_2=CH–CH_2–	H	H	Br	153	C	84
75	4-CH_3–C_6H_4–	3-CH_3–C_6H_4–CH_2–	H	H	Br	195	C	82
76	4-CH_3–C_6H_4–	4-Cl–C_6H_4–CH_2–	H	H	Cl	183	C	92
77	4-CH_3–C_6H_4–	4-NO_2–C_6H_4–CH_2–	H	H	Br	204	C	85
78	4-CH_3–C_6H_4–	4-Cl–C_6H_4–CH_2–	CH_3	H	Cl	200	D	90

[a] Crystallization solvents. A, 2-methoxyethanol; B, *n*-propanol; C, acetonitrile; D, ethanol; E, 2-propanol; F, methanol; G, dimethylformamide.

These new compounds are related structurally to the arylideneiminoimidazoles **A**, of which they can be considered the linear precursors.

2. Chemistry

Compounds **B** have been synthesised by alkylating the appropriate thiosemicarbazide, obtained by reaction of the suitable isothiocyanate with hydrazine in methanol, and condensation of the resulting product with an aldehyde or a ketone.

The structures of the synthesised compounds are in agreement with the analytical, spectroscopic (IR; ^{1}H NMR) and mass spectrometric data.

The IR spectra in CCl_4 solution of the *N*-unsubstituted isothiosemicarbazones ($R_1 = H$, Scheme 1), exhibit characteristic absorption bands ranging between 3500 and 3380 cm^{-1}, typical of amidic structure [12].

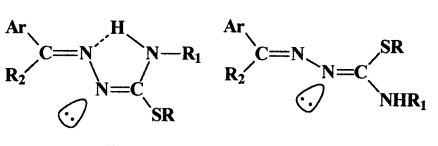
Their solid-state IR spectra show two characteristic absorption bands between 3280 and 3050 cm^{-1}. The ^{1}H NMR spectra of the arylideneisothiosemicarbazones bearing a substituent on the amine nitrogen atom (NHR_1), recorded in deuterated chloroform and in dimethylsulfoxide for the free bases and for the hydrohalogenated compounds, respectively, exhibit two different clusters of signals.

Two singlets and two doublets can be observed in the spectra of those compounds bearing a methyl or a methylene group on the sulfur atom with a ratio of 7:3. The two doublets originate from the NH–CH_3 protons and are reduced to singlets by treatment with deuterated water. A rationale of this behaviour could be given assuming that these compounds present two different diastereoisomers, *E* and *Z*. These two configurations are probably constrained around the C=N[2] double bond.

Further evidence is given by the spectra of those compounds where R_2 is a methyl group (Scheme 1). In this case the methyl signal is a singlet, which seems to exclude the formation of two diastereoisomers around the C=N[1] bond. Only one cluster of signals can be observed for those compounds that are not substituted on the N terminus ($R_1 = H$).

Although the chemical shifts are comparable to those of the substituted derivatives, this cluster is observed at lower fields. Within the two diastereoisomers, the one that shows chemical shifts at lower fields enjoys the stabilising effect of the intramolecular hydrogen bond between the secondary nitrogen atom and the one bearing the arylidene substituent (Scheme 2).

The diastereoisomers were separated by column chromatography, performed on silica gel, eluent ethanol–petroleum ether (3:1).



Scheme 2.

Table 2
MIC values of some of the most significant arylideneisothiosemicarbazones

Comp.	Ar	R	R_1	R_2	X	*S. aureus*	*S. epidermidis*	*B. subtilis*	*B. thurigensis*	*S. faecalis*	*S. agalactiae*
1	2-(5-NO_2–C_4H_2O)–	*n*-C_4H_9	CH_3	H	Br	6.25	6.25	6.25	6.25	50	50
2	2-(5-NO_2–C_4H_2O)–	CH_2=CH–CH_2–	CH_3	H	Br	12.5	6.25	12.5	6.25	6.25	100
4	2-(5-NO_2–C_4H_2O)–	3-CH_3–C_6H_4–CH_2–	H	H	Br	3.13	1.56	3.13	3.13	25	25
5	2-(5-NO_2–C_4H_2O)–	4-Cl–C_6H_4–CH_2–	CH_3	H	Cl	50	1.56	12.5	1.56	>100	>100
a [a]	2-(5-NO_2–C_4H_2O)–	3,4-Cl_2–C_6H_3–CH_2–	H	H	Cl	1.56	0.39	0.78	0.78	3.13	1.56
b [b]	4-Cl–C_6H_4–	4-Cl–C_6H_4–CH_2–	H	H	Cl	6.25	6.25	6.25	6.25	100	6.25
15	4-Cl–C_6H_4–	4-Cl–C_6H_4–CH_2–	H	CH_3	Cl	25	12.50	25	25	50	50
c [a]	4-Cl–C_6H_4–	3,4-Cl_2–C_6H_3–CH_2–	H	H	Cl	6.25	3.13	3.13	6.25	100	50
19	3-Cl–C_6H_4–	*n*-C_4H_9	H	H	Br	25	50	25	25	>100	25
21	3-Cl–C_6H_4–	3-CH_3–C_6H_4–CH_2–	H	H	Br	25	>100	25	50	>100	12.50
22	3-Cl–C_6H_4–	4-Cl–C_6H_4–CH_2–	H	H	Cl	12.50	12.50	12.50	12.50	>100	12.50
25	3,4-Cl_2–C_6H_3–	*n*-C_4H_9	H	H	Br	12.50	12.50	12.50	12.50	>100	12.50
26	3,4-Cl_2–C_6H_3–	*n*-C_4H_9	H	H	Br	50	100	50	50	25	25
28	3,4-Cl_2–C_6H_3–	4-Cl–C_6H_4–CH_2–	H	H	Cl	6.25	3.12	6.25	6.25	>100	12.50
30	3,4-Cl_2–C_6H_3–	CH_2=CH–CH_2–	CH_3	H	Br	25	25	50	25	100	25
36	3-Br–C_6H_4–	*n*-C_4H_9–	H	H	Br	50	>100	>100	50	>100	50
39	3-Br–C_6H_4–	4-Cl–C_6H_4–CH_2–	H	H	Cl	6.25	12.50	12.50	6.25	>100	25
41	4-F–C_6H_4–	*n*-C_4H_9	H	H	Br	100	100	50	50	>100	50
43	4-F–C_6H_4–	3-CH_3–C_6H_4–CH_2–	H	H	Br	50	>100	50	50	>100	100
53	3-F–C_6H_4–	*n*-C_4H_9	H	H	Br	100	100	100	100	>100	50
55	3-F–C_6H_4–	3-CH_3–C_6H_4–CH_2–	H	H	Br	25	>100	50	50	>100	25
56	3-F–C_6H_4–	4-Cl–C_6H_4–CH_2–	H	H	Cl	25	>100	25	25	>100	25
58	4-CF_3–C_6H_4–	*n*-C_4H_9	H	H	Br	>100	100	25	>100	100	100
66	4-HO–C_6H_4–	4–Cl–C_6H_4–CH_2–	H	H	Cl	50	100	25	50	>100	>100

[a] Ref. [5].
[b] Ref. [13].

3. Microbiology

Microbiological tests were performed on the following strains: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Bacillus thurigensis*, *Streptococcus faecalis*, *Streptococcus agalactiae* and *Candida albicans*.

The determination of minimum inhibitory concentration (MIC), in μg/ml of product, was carried out according to the previously described [3–5] broth microdilution method. The compounds were dissolved in dimethylsulfoxide and then diluted in the test medium, in order to obtain the required concentration (ranging between 0.39 and 100 μg/ml). The cultures were obtained on Casitone broth (5 g/l yeast extract Difco, 5 g/l Casitone Difco, 5 g/l glucose) and on Tryptose Agar for the fungi and the bacteria, respectively.

4. Structure–activity relationships

Examination of the microbiological data together with the structural properties of the synthesised compounds allows some considerations on structure–activity relationships. Higher activity was observed for compounds bearing the 5-nitrofuryl group.

Although important, the presence of this substituent is not essential for antimicrobial activity; a significant activity is also evidenced by those alkylisothiosemicarbazones bearing a benzyl substituent, either mono- or dihalogenated, instead of the nitrofuryl group.

The most potent compounds are those where a chlorine atom is present on the benzyl ring, particularly in the *para* and/or *meta* position. The substitution of the chlorine atom with other halogens leads to a decrease of the antimicrobial activity (Cl > Br > F).

Higher MIC values were also observed upon the introduction of a second halogen atom in the arylidene ring, with the exception of the 3,4-dichlorobenzylidene derivative with appropriate substitutions on the sulfur atom (compound **27**). This compound exhibits MIC values of 3.12 μg/ml for *Staphylococcus epidermidis* and 6.25 μg/ml for *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus thurigensis*.

The introduction of other substituents such as NO_2, CH_3O, CH_3, OH and CF_3, always leads to a dramatic increase of the MIC values and, in most cases, to complete loss of activity. The activity is affected strongly by the nature of the thioether substituent, standing all the other structural conditions.

Moreover, when R (Scheme 1) is an alkyl group the biological response, depending on the nature of the tested bacteria strains, is not influenced significantly. However, for all the tested arylideneisothiosemicarbazones, the longer the alkyl chain is, the higher the activity, reaching the maximum when R = n-C_4H_9.

An increase of the antibacterial power has been observed when R is a benzyl or, even better, a halo-substituted benzyl group. This increase is particularly evident in the case of *bacilli* and *staphylococci*, but is observed, to a lower extent, for *streptococci*.

For the above agents, substitution of the halogen atom with a methyl or a nitro group does not impair the biological activity. An almost complete loss of activity has been observed when a methoxy group replaces the halogen atom. The substitution of a hydrogen atom with an alkyl group on the amine nitrogen (R_1 = alkyl) generally leads to a decreased antimicrobial activity. Also the substitution of the arylidene hydrogen with an alkyl group (R_2 = alkyl) leads to a dramatic decrease of the activity.

In general, we observe that most of the synthesised compounds are more potent against *S. aureus*, *S. epidermidis*, *B. subtilis* and *B. thurigensis* than against *S. agalatiae* and *S. faecalis*.

The antimicotic activity of some of the novel compounds was evaluated on strains of *C. albicans* and *C. tropicalis*. However, the minimum fungicidal concentration (MFC) was observed at relatively high concentrations for all tested compounds. MIC values of some of the most significant arylidenisothiosemicarbazones are reported in Table 2.

5. Conclusions

This new class of compounds, structurally related to the arylideneamino-2-mercaptoimidazoles, has shown both a good antibacterial and fungicidal activity. It has been observed that the introduction of the thioether group leads to a remarkable enhancement of the biological activity, with MIC values comparable to those of the imidazole derivatives.

Moreover, with respect to the mercaptoimidazoles, these new compounds can be more easily synthesised in good yields.

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