APPLIED MICROBIOLOGY, Apr. 1968, p. 582-587
Vol. 16, No. 4

Printed in U.S.A.

Antifungal Activity of Isothiocyanates and Related Compounds

III. Derivatives of Biphenyl, Stilbene, Azobenzene, and Several Polycondensed Aromatic Hydrocarbons

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Received for publication 28 November 1967

This paper presents the results of a study on the antifungal activity of isothiocyanates—derivatives of biphenyl (group "A"), of stilbene ("B"), of azobenzene and benzeneazonaphthalene ("C"), of naphthalene ("D"), and of further polycondensed aromatic hydrocarbons ("E"). From a total of 48 investigated compounds, antifungal activity was observed only in A and D group compounds. B, C, and E group derivatives are extremely insoluble in water, and the molecules are very large; as a result, they probably cannot pass into spores or mycelium of fungi. Thus, the —NCS group cannot manifest its reactivity.

As a continuation of our studies on the antifungal activity of natural and synthetic isothiocyanates (10, 12, 13), the present paper deals with the activity of derivatives which may be divided into five groups.

In the first group ("A"), there are compounds of the type shown in Fig. 1 and 2, where X is a direct bond between two benzene rings or a bond by means of —O—, —CO—, or —CO—O— groups. The alternative possibility concerns only the two isothiocyanate derivatives studied, where R represents an aromatic ring with an —NCS radical bound by means of the direct bond or the pyridine ring bound by means of the —CO—O— group.

The second group ("B") is represented by derivatives of stilbene (Fig. 3), i.e., isothiocyanates of stilbene and substituted stilbenes.

Group "C" comprises derivatives of azobenzene (Fig. 4), dimethylaminoazobenzene (Fig. 5), and benzeneazonaphthalene (Fig. 6), where R is methyl group or hydrogen.

The next group ("D") is represented by isothiocyanates, arylmethylisothiocyanates, and diisothiocyanates of naphthalene.

Finally, group "E" comprises derivatives of anthracene, phenanthrene, chrysene, pyrene, anthraquinone, and fluorene.

Several group A compounds display an outstanding antiyeast and antihelmintic activity (6, 8, 9, 22). Group B and C isothiocyanates were synthetized from corresponding amines which are known as carcinogens or mutagens (21). A detailed description of the physical and chemical properties of group C derivatives has been presented in previous papers (1, 2, 4). From the derivatives of group D, 1-naphthylisothiocyanate represents a long-known fungicide. During recent years, this compound has been of interest because it provokes experimental jaundice in laboratory animals (20, 24). Its analogue, 2-naphthylisothiocyanate, derived from the carcinogen 2-naphthylamine, showed carcinostatic activity in experiments with the Ehrlich ascites carcinoma and in skin carcinoma provoked by 3,4-benzpyrene in mice (7, 23). 2-Naphthylisothiocyanate displays a higher activity against yeasts and yeast-like organisms than does the 1-naphthyl analogue (22).

MATERIALS AND METHODS

Compounds. Group A compounds (I–VII, Table 1; Fig. 7–13) were synthesized by procedures reported previously (6, 14, 16). The synthesis of group B derivatives (VIII–XVI, Table 2) (21), group C (XVII–XXVIII, Table 3) compounds (1, 2, 4), and group D (XXIX–XXXIX, Table 4) compounds have been described (3, 18, 19). The synthesis and properties of isothiocyanates of polycondensed aromatic hydrocar-

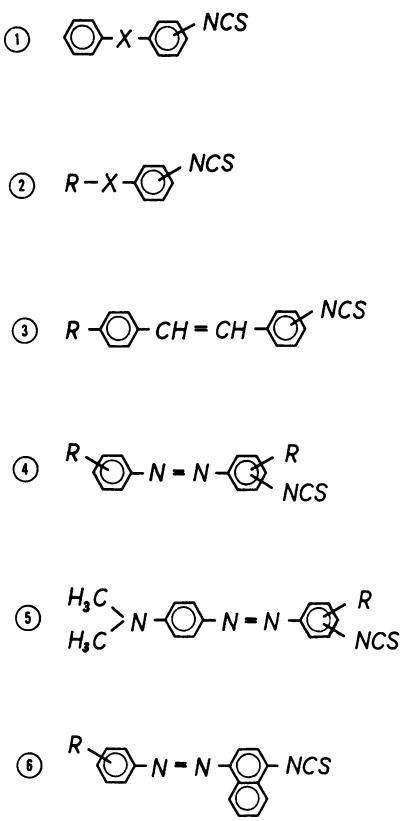

FIG. 1–6. *Isothiocyanate derivatives. Figures 1 and 2 represent the structure of group A compounds; Fig. 3 shows the structure of stilbene, from which group B derivatives are obtained; Fig. 4 (azobenzene), Fig. 5 (dimethylaminoazobenzene), and Fig. 6 (benzeneazonaphthalene) represent sources of group C derivatives.*

bons (group E, XL–XLVIII, Table 5) were described in previous reports (15, 17–19). Isothiocyanates II, V–XVI, XVIII–XXVIII, XXXII–XXXIV, and XXXVII–XLVIII represent new compounds prepared in our laboratories.

Antifungal activity. All compounds were tested on *Aspergillus niger* 11/13, *Penicillium cyclopium* 11/17, and *Rhizopus oryzae* 5/1; 1-naphthyl- and 2-naphthylisothiocyanates were also tested on 13 other strains of fungi. The source of strains, methods of cultivation, and determination of antifungal activity were reported elsewhere (12, 13).

RESULTS AND DISCUSSION

Of the isothiocyanates studied, definite antifungal activity was detected only with the group A and D compounds (Tables 1 and 4). All substances of group A may be considered as substituted derivatives of phenylisothiocyanate, in contradistinction to previously studied compounds of this group (13); however, they are characterized by the great size of the substituent. Consequently, they are mostly of poor water solubility. For phenylisothiocyanate, the solubility value is 6.65×10^{-4} moles/liter; for

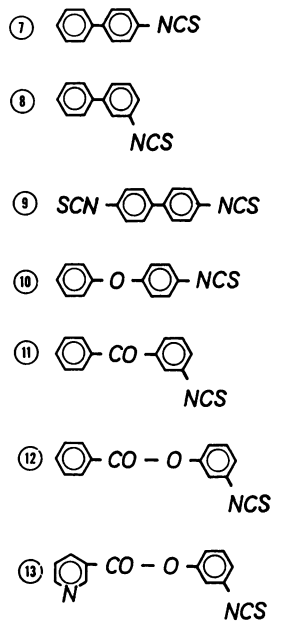

FIG. 7–13. *Group A derivatives I–VII (see Table 1).*

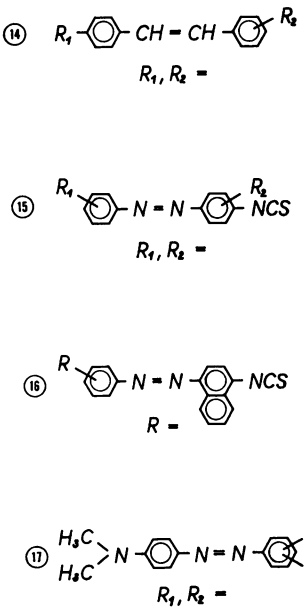

FIG. 14. *Structure of group B derivatives (compounds VIII–XVI). For R_1 and R_2 substituents, see Table 2.*

FIG. 15–17. *Compounds XVII and XVIII (Fig. 15), XIX–XXI (Fig. 16), and XXII–XXVIII (Fig. 17). The indicated substituents are given in Table 3.*

TABLE 1. *Antifungal activity of group A compounds against Aspergillus niger, Penicillium cyclopium, and Rhizopus oryzae as compared with allyl- and phenylisothiocyanate*

No.	Structural formula	Mol wt	The ED_{50} and ED_{100} values (moles/liter) and activity after 14 days of incubation[a]			RE[b]	RE[c]
			A. niger	*P. cyclopium*	*R. oryzae*		
I	Fig. 7	211.270	32×10^{-6} / 30×10^{-5} > C	34×10^{-6} / 22×10^{-5} D	16×10^{-6} / 60×10^{-6} D	2.2	0.3
II	Fig. 8	211.270	ca. 20×10^{-5} / $>15 \times 10^{-4}$ > B		ca. 10×10^{-5} / $>15 \times 10^{-4}$ > B	<0.4	<0.07
III	Fig. 9	268.340	$>15 \times 10^{-4}$ / $>15 \times 10^{-4}$ > B	$>15 \times 10^{-4}$ / $>15 \times 10^{-4}$ > B	$>15 \times 10^{-4}$ / $>15 \times 10^{-4}$ > B	<0.4	<0.07
IV	Fig. 10	227.270	50×10^{-6} / 13×10^{-4} > B		28×10^{-5} / 50×10^{-5} B	0.5	0.08
V	Fig. 11	239.280	46×10^{-6} / 86×10^{-6} D		ca. 50×10^{-5} / ca. 13×10^{-4} B	7.6	1.2
VI	Fig. 12	255.280	25×10^{-5} / 56×10^{-5} C		ca. 30×10^{-5} / ca. 10×10^{-4} > C	1.1	0.1
VII	Fig. 13	256.270	21×10^{-6} / 42×10^{-6} D		50×10^{-6} / 11×10^{-5} D	15.7	2.6

[a] For each compound, a corresponding ED_{50} value is given, followed below by the ED_{100} value; ED_{50} and ED_{100} values for *A. niger* were assessed after 4 days, and, in the other two fungi, after 6 days of incubation. Block letters designate the lowest of the tested concentrations capable of completely suppressing fungal growth after 14 days (in moles per liter): A, 3×10^{-7}; B, 1.5×10^{-7}; C, 7.5×10^{-6}; D, 37.5×10^{-6}; E, 7.5×10^{-5}; F, 3.75×10^{-5}.

[b] RE = relative effectiveness of isothiocyanates compared with that of allylisothiocyanate, i.e., the ED_{100} values ratio of both compounds (*A. niger*); ED_{100} of allylisothiocyanate = 66×10^{-5} moles/liter (12).

[c] RE = relative effectiveness of isothiocyanates compared with that of phenylisothiocyanate; ED_{100} of phenylisothiocyanate for *A. niger* = 11×10^{-5} moles/liter (13).

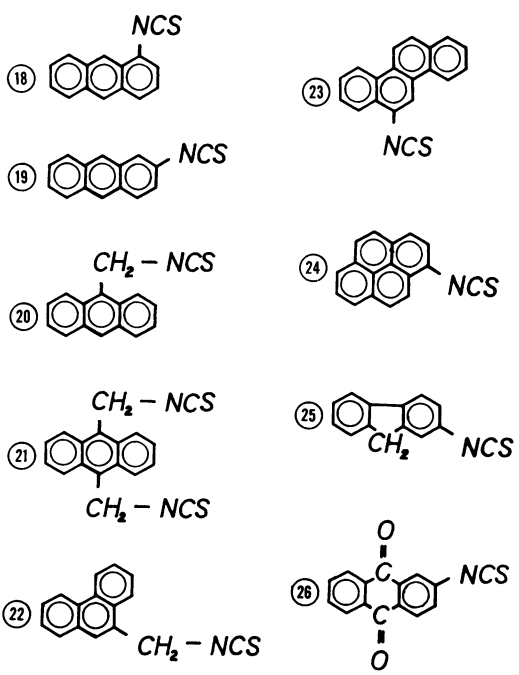

FIG. 18–26. *Compounds XL–XLVIII (group E) are represented by Fig. 18–26, respectively. At the highest concentration tested (37.5×10^{-5} moles/liter), compounds XL–LVI were ineffective against A. niger, P. cyclopium, and R. oryzae. The ED_{50} of compound XLVII was 50×10^{-5} moles/liter; the ED_{50} of compound XLVIII was 65×10^{-5} moles/liter (against A. niger).*

TABLE 2. *Group B compounds tested for antifungal activity*[a]

No.	R_1, R_2 (Fig. 14)
VIII	H, 4-NCS
IX	H, 3-NCS
X	H, 2-NCS
XI	Cl, 4-NCS
XII	Br, 4-NCS
XIII	CH_3, 4-NCS
XIV	O—CH_3, 4-NCS
XV	NO_2, 4-NCS
XVI	$N(CH_3)_2$, 4-NCS

[a] On account of their poor solubility, these compounds were tested only up to concentrations not influencing the growth of *A. niger*, *P. cyclopium*, and *R. oryzae*. With compounds XII, XIV, and XVI, the concentration limit was 7.5×10^{-5} moles per liter; for all other compounds, 37.5×10^{-5} moles per liter.

phenoxyphenylisothiocyanate, 0.17×10^{-4} moles/liter; and for 4-biphenylylisothiocyanate, 0.14×10^{-4} moles/liter, determined at 25 C (11). Compared with other isothiocyanates, the difference in

TABLE 3. *Group C derivatives tested for antifungal activity*[a]

No.	R_1, R_2 (Fig. 15)	No.	R (Fig. 16)	No.	R_1, R_2 (Fig. 17)
XVII	H, H	XIX	4-CH_3	XXII	4-NCS, H
XVIII	2-CH_3, 3-CH_3	XX	3-CH_3	XXIII	4-NCS, 3-CH_3
		XXI	2-CH_3	XXIV	4-NCS, 2-CH_3
				XXV	3-NCS, H
				XXVI	3-NCS, 4-CH_3
				XXVII	3-NCS, 6-CH_3
				XXVIII	2-NCS, 3-CH_3

[a] The highest tested concentration of compounds XVII and XXV was 3.75×10^{-5} moles per liter; of compound XVIII, 7.5×10^{-5}; of others, 0.75×10^{-5} moles per liter.

TABLE 4. *Antifungal activity of isothiocyanates of naphthalene (group D compounds) against Aspergillus niger, Penicillium cyclopium, and Rhizopus oryzae, and comparison with phenylisothiocyanate*

No.	Substituents	Mol wt	ED_{50} and ED_{100} values (moles/liter) and activity after 14 days of incubation[a]			RE[b]
			A. niger	*P. cyclopium*	*R. oryzae*	
XXIX	1-NCS	185.234	61×10^{-7} 85×10^{-6} C	38×10^{-7} 91×10^{-6} C	11×10^{-6} 57×10^{-6} E	1.2
XXX	2-NCS	185.234	13×10^{-6} 63×10^{-6} D	18×10^{-6} 89×10^{-6} C	27×10^{-6} 19×10^{-5} D	1.7
XXXI	1-NCS, 2-Cl, 4-Cl	254.132	B	B	B	
XXXII	1-CH_2—NCS	199.260	10×10^{-6} 89×10^{-6} D	16×10^{-6} 63×10^{-6} D	45×10^{-6} 36×10^{-5} > C	1.2
XXXIII	1-CH_2—NCS, 5-NCS	256.330	$>38 \times 10^{-5}$ $>38 \times 10^{-5}$ > D	$>38 \times 10^{-5}$ $>38 \times 10^{-5}$ > D	$>38 \times 10^{-5}$ $>38 \times 10^{-5}$ > D	<0.2
XXXIV	1-CH_2—NCS, 4-CH_2—NCS	270.356	$>75 \times 10^{-5}$ $>75 \times 10^{-5}$ > C	$>75 \times 10^{-5}$ $>75 \times 10^{-5}$ > C	$>75 \times 10^{-5}$ $>75 \times 10^{-5}$ > C	<0.1
XXXV	1-CH_2—NCS, 5-CH_2—NCS	270.356	$>38 \times 10^{-5}$ $>38 \times 10^{-5}$ > D	$>38 \times 10^{-5}$ $>38 \times 10^{-5}$ > D	$>38 \times 10^{-5}$ $>38 \times 10^{-5}$ > D	<0.2
XXXVI	1-Br, 2-NCS	264.142	B	C	B	
XXXVII	2-NCS, 6-SO_3Na	287.283	10×10^{-5} 45×10^{-5} > B	> B	> B	0.2
XXXVIII	2-NCS, 6-SO_3Na, 8-OH	303.283	10×10^{-5} 45×10^{-5} > B	> B	> B	0.2
XXXIX	2-NCS, 5-OH, 7-SO_3Na	303.283	16×10^{-5} 10×10^{-4} > B	> B	> B	0.1

[a] See footnote *a*, Table 1.
[b] See footnote *c*, Table 1.

solubility between phenylisothiocyanate and isothiocyanate derivatives of biphenyl is considerably greater than the difference in their reactivity (11, 25).

Particularly, the solubility of group B, C, and E derivatives in water and in organic solvents employed for antifungal activity tests (ethyl alcohol, diethyleneglycol, monoethylether, or ethyleneglycol) was so low that estimation of activity was possible only up to concentrations (shown in Tables 2, 3, and 6) which did not, in fact, inhibit the growth of tested fungi. Thus, these compounds are rather uninteresting in terms of antifungal activity. However, some of these extremely insoluble compounds, in very low concentrations, definitely influence the glycolysis of Ehrlich ascites tumor cells (21), and their influence on animal cells is being studied at the present time. Based on the present knowledge of subcellular distribution of isothiocyanates in bacteria, fungi, yeast, and animal cells, it may be assumed that neither group B nor group C derivatives can pass the cell wall of *A. niger* or of yeasts (5, 9).

Table 4 comprises results describing the antifungal activity of naphthalene derivatives. The most active were the 1- and 2-naphthyl- as well as the 1-(naphthylmethyl)-isothiocyanates. Somewhat surprising is the relatively low activity of all

TABLE 5. *Activity of two isothiocyanates on 16 strains of fungi*

Isothiocyanate	Tested strains[a]	ED_{100} (moles per liter)				
		$<10^{-5}$	10^{-5} to 10^{-6}	10^{-6} to 5×10^{-6}	5×10^{-6} to 10^{-7}	$>10^{-7}$
1-Naphthyl-	1–11, 13–16	11	1–3, 5–10, 16	4, 13–15		
2-Naphthyl-	1–16	16	1, 2, 5–11, 15	3, 4, 14	13	12

[a] Individual strains are numbered as follows (numbers in parentheses indicate days of incubation): 1, *Aspergillus niger* (4); 2, *Penicillium cyclopium* (6); 3, *Rhizopus oryzae* (6); 4, *A. flavus* (6); 5, *A. oryzae* (5); 6, *P. chrysogenum* (5); 7, *P. brevicompactum* (5); 8, *Cladosporium herbarum* (6); 9, *Trichoderma viride* (7); 10, *Alternaria tenuis* (7); 11, *Monilia sitophila* (7); 12, *Trichophyton gypseum asteroides* (30); 13, *Cytospora* sp. (14); 14, *Schizophyllum commune* (14); 15, *Fusarium* sp. (14); 16, *Cephalothecium roseum* (14).

diisothiocyanates tested, i.e., 1,4- and 1,5-bis-(isothiocyanatomethyl)-naphthalene and 1-(isothiocyanatomethyl)-naphthalene, in which the hydrogen in position 5 is substituted by the —NCS group. Since all other substituted naphthylisothiocyanates are less active, compared with unsubstituted derivatives, it may be concluded that the introduction of a further substituent besides the —NCS or —CH_2—NCS group on the naphthalene ring usually results in a decrease of activity This decrease is due to a change in the molecule size, which relates to the solubility of the compound. It must be stressed that such conclusions are valid only in the fungi and yeasts and not in bacteria, against which even some of the arylmethylisothiocyanates (Fig. 18–26) display limited activity. These isothiocyanates of polycondensed aromatic hydrocarbons showed practically no antifungal activity.

Table 5 presents a survey of the activity of 1-naphthyl- and 2-naphthylisothiocyanates on 16 strains of fungi. There are few differences, either in spectrum or in the degree of activity, between the two compounds.

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