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Short communication

Synthesis, antifungal and nematocidal activities of thioureines with an aminoester sequence

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Summary — Twenty-three arylthioureines bearing β-alanine or γ-aminobutyric alkyl ester chains were synthesized for *in vitro* screening toward 44 strains of fungi and 2 genera of nematodes. The nitro derivatives were the most potent compounds against *Aspergillus* and *Candida* strains. Ester chains increase activity against the filarial worm *Molinema dessetae*. Twelve compounds have $EC_{50} < 40$ μg/ml. However, the anthelmintic potency is weak compared with tetramisole.

antifungal activity / anthelmintic / thioureine

Introduction

Since the discovery of anthelmintic tetramisole **1** (fig 1), a number of analogues have been synthesized, particularly 6-(3-aminophenyl) derivatives. The corresponding thioureas have been reported to possess broad spectrum activity against nematodes, trematodes and cestodes [1]. Many authors have reported anthelmintic activity for molecules bearing the thiourea moiety [2–4]. Walchshofer *et al* [5] have studied structural parameters for thiazolyl ureas and thioureas and noted the influence of lipophilicity on anthelmintic activity. In a factorial analysis of the correspondence between chemical structure and antiparasitic activity, Doré *et al* [6] revealed the potential of thioureas as antiparasitic agents. Numerous sulfur-containing molecules with divalent sulfur attached to carbon atoms have *in vitro* fungistatic action, *eg*, dithiocarbamates and thiurams [7]. Thus, thioureines are analogues of antifungal structures and were tested against fungi.

On basis of these results, we previously studied aminothiazolines **2** and aminothiazines **3** [8], which are open analogues of anthelmintic tetramisole **1** (fig 1), and then thioureines **4** and **5** bearing a β-alanine or γ-aminobutyric moiety [9]. Such structures are GABA-like compounds (GABA = γ-aminobutyric acid) and potential anthelmintic agents, because GABA is an inhibitory transmitter for the neuromuscular system of nematodes [10]. GABA itself is not very potent and the moderate anthelmintic and antifungal activities of compounds **4** and **5** were assigned to their weak lipophilicity. In a continuation of this work, we report here the synthesis and activities of some more lipophilic esters of thioureines with the same β-alanine or γ-amino butyric sequences.

Chemistry

Compounds **9** were obtained in four steps from aromatic amines through condensation of corresponding isothiocyanates **8** with the appropriate aminoesters **6** or **7**, following scheme 1. Isothiocyanates **8** were prepared as previously described by Hodgkins *et al* [11]; aminoesters of β-alanine or GABA were obtained in the usual manner. Physicochemical and NMR data for compounds **9a–w** are given in tables I and II.

Results

Antifungal activities

All compounds were screened *in vitro* against an array of clinical isolates of 35 strains of *Candida* and one reference strain *C albicans* AFNOR ATCC 2094, and

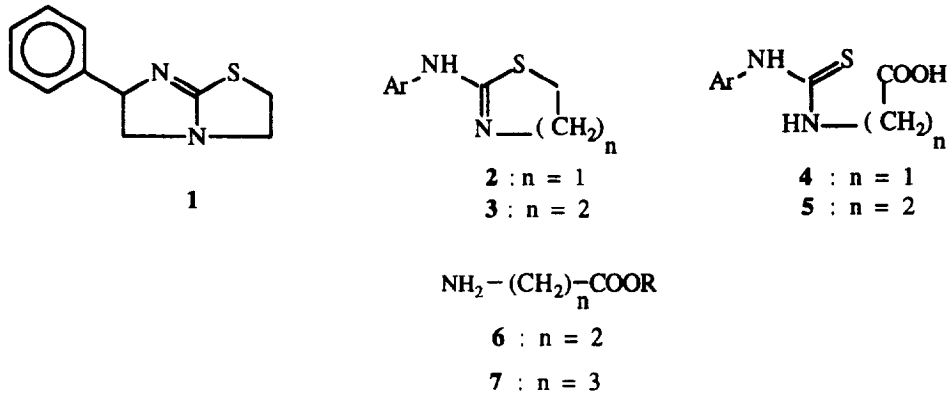
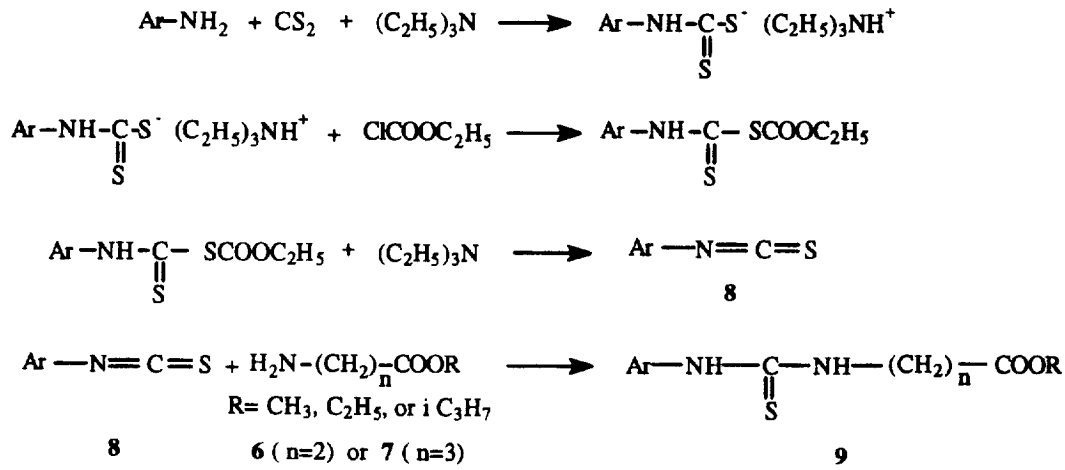


Fig 1. Structures of compounds 1–7.



Scheme 1.

eight strains of opportunistic fungi (*Aspergillus fumigatus, A flavus, A niger* and *Scopulariopsis brevicaulis*). The results are reported in tables III and IV. When the minimum inhibitory concentrations (MIC) are close for different strains of the same species, we report a median value.

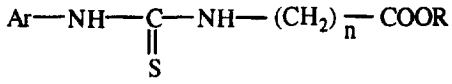
Anthelmintic activities

The same compounds were tested against infective larvae of an intestinal parasite of rats, *Nippostrongylus brasiliensis*, and against infective larvae of a filaria, *Molinema dessetae*. These two tests were chosen because they tend to detect *in vitro* activities that are generally confirmed *in vivo* [6–8]. The results (EC_{50} in μg/ml) are reported in table V.

Discussion

All the antifungal activities were moderate, except for **9d** (4 μg/ml < MIC < 8 μg/ml for all strains of *C albicans* and 2 μg/ml < MIC < 8 μg/ml for all strains of *Aspergillus*). The two nitro compounds **9s** and **9t** were equipotent (4 μg/ml < MIC < 16 μg/ml for all strains of *Candida* in Casitone (CAS) medium and for all strains of *Aspergillus*, in both yeast nitrogen-based glucose (YNBG) and CAS medium) and their MIC values were less than econazole for all strains of *Candida*. These methyl and isopropyl esters indicate that the nature of the ester group is not determinant for activity. Moreover, three esters of the same molecule **9a–c** had differing antifungal activity (**9a** < **9b** < **9c**); **9d** is very active whereas the corresponding ethyl

Table I. Physicochemical data for compounds **9a–w**.

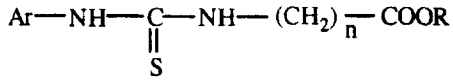


Compound	*Ar*	*n*	*R*	*Formula*	*MW*	*Mp (°C)*	*Yield (%)*
9a	Phenyl	2	Methyl	$C_{11}H_{14}N_2O_2S$	238.2	72–73	80
9b	Phenyl	2	Ethyl	$C_{12}H_{16}N_2O_2S$	252.2	108–109	40
9c	Phenyl	2	*i*-Propyl	$C_{13}H_{18}N_2O_2S$	266.2	111–113	72
9d	*p*-Anisyl	2	Methyl	$C_{12}H_{16}N_2O_3S$	268.2	135	78
9e	*p*-Anisyl	2	Ethyl	$C_{13}H_{18}N_2O_3S$	282.3	138–139	46
9f	*p*-Anisyl	2	*i*-Propyl	$C_{14}H_{20}N_2O_3S$	296.3	95–97	81
9g	*p*-Nitrophenyl	2	Methyl	$C_{11}H_{13}N_3O_4S$	283.3	153–154	38
9h	3,4-Dimethoxyphenyl	2	Methyl	$C_{13}H_{18}N_2O_4S$	298.3	123–124	51
9i	3,4-Dimethoxyphenyl	2	Ethyl	$C_{14}H_{20}N_2O_4S$	312.3	138–140	43
9j	3,4-Dimethoxyphenyl	2	*i*-Propyl	$C_{15}H_{22}N_2O_4S$	326.3	126	50
9k	2-Benzodioxanyl	2	Methyl	$C_{13}H_{16}N_2O_4S$	296.2	130	79
9l	2-Benzodioxanyl	2	*i*-Propyl	$C_{15}H_{20}N_2O_4S$	324.3	114	40
9m	Phenyl	3	Methyl	$C_{12}H_{16}N_2O_2S$	252.2	131–132	59
9n	Phenyl	3	Ethyl	$C_{13}H_{18}N_2O_2S$	266.2	98–101	40
9o	Phenyl	3	*i*-Propyl	$C_{14}H_{20}N_2O_2S$	280.2	107–109	75
9p	*p*-Anisyl	3	Methyl	$C_{13}H_{18}N_2O_3S$	282.3	112–114	58
9q	*p*-Anisyl	3	Ethyl	$C_{14}H_{20}N_2O_3S$	296.3	96	78
9r	*p*-Anisyl	3	*i*-Propyl	$C_{15}H_{22}N_2O_3S$	310.3	105–108	57
9s	*p*-Nitrophenyl	3	Methyl	$C_{12}H_{15}N_3O_4S$	297.3	155–157	53
9t	*p*-Nitrophenyl	3	*i*-Propyl	$C_{14}H_{19}N_3O_4S$	325.3	160–162	61
9u	3,4-Dimethoxyphenyl	3	*i*-Propyl	$C_{16}H_{24}N_2O_4S$	340.3	112	86
9v	2-Benzodioxanyl	3	Methyl	$C_{14}H_{18}N_2O_4S$	310.3	122	45
9w	2-Benzodioxanyl	3	*i*-Propyl	$C_{16}H_{22}N_2O_4S$	338.3	120–121	40

and isopropyl esters **9e** and **9f** have similar activities; and esters **9h–j** of the same molecule have comparable levels of activity. It is thus impossible to correlate activity with the nature of the ester group. The influence of the nature of the aminoester group did not prove any superiority of β-alanine over the γ-aminobutyric moiety. More generally, the comparison of these results with the activities of the corresponding acids [9] did not prove any superiority of esters.

In contrast, esterification always enhances anthelmintic activity (mainly filaricid). The EC_{50} values were < 50 μg/ml for 14 compounds against *M dessetae* at 7 d, whereas the acid analogues of **9a** and **9g** and the two most active compounds **9p** and **9s** were completely ineffective [2]. Examination of the results did not prove any superiority of β-alanine over the γ-aminobutyric moiety.

Table II. NMR data for **9a–w** ($CDCl_3$ or $CDCl_3/CD_3OD$[a] at 60 MHz).



Compound	*Aromatic protons*	*ArNH (s, 1H), NHCH₂ (t, 1H)*	*NHCH₂ (m, 2H), CH₂CO (t, 2H), CH₂CH₂CH₂ (m, 2H)*	*Ester R*[b]	*Others*
9a	7.40 (m, 5H)	8.66, 6.90	3.97, 2.83	3.67 (s, 3H)	–
9b	7.33 (m, 5H)	7.92, 6.83	3.87, 2.66	4.15 (q, 2H), 1.18 (t, 3H)	–
9c	7.30 (m, 5H)	8.87, 6.90	3.93, 2.63	4.91 (h, 1H), 1.17 (d, 6H)	–
9d	7.19 (d, 2H), 6.92 (d, 2H)	8.17, 6.63	3.96, 2.68	3.65 (s, 3H)	3.83 (s, 3H, CH_3O)
9e	7.37 (d, 2H), 6.93 (d, 2H)	7.90, 6.88	3.92, 2.73	4.16 (q, 2H), 1.37 (t, 3H)	3.83 (s, 3H, CH_3O)
9f	7.25 (d, 2H), 6.88 (d, 2H)	8.07, 6.74	3.95, 2.70	4.89 (h, 1H), 1.20 (d, 6H)	3.82 (s, 3H, CH_3O)
9g[a]	8.15 (d, 2H), 7.75 (d, 2H)	exch, exch	3.93, 2.75	3.39 (s, 3H)	–
9h	6.85–7.01 (m, 3H)	8.42, 6.85	3.86, 2.76	3.93 (s, 3H)	3.85–3.88 (2s, 6H, 2 CH_3O)
9i	6.82–6.95 (m, 3H)	8.40, 6.90	3.83, 2.75	4.15 (q, 2H), 1.25 (t, 3H)	3.85 (2s, 6H, 2 CH_3O)
9j	6.83–6.97 (m, 3H)	8.55, 6.45	3.96, 2.71	5.08 (h, 1H), 1.23 (d, 6H)	3.84 (2s, 6H, 2 CH_3O)
9k	6.85–6.94 (m, 3H)	8.18, 6.75	3.83, 2.63	3.65 (s, 3H)	4.40 (m, 4H, OCH_2CH_2O)
9l	6.90–6.98 (m, 3H)	8.05, 6.48	4.05, 2.43	5.02 (h, 1H), 1.25 (d, 6H)	4.38 (m, 4H, OCH_2CH_2O)
9m	7.35 (m, 5H)	8.77, 6.67	3.68, 2.66, 1.93	3.63 (s, 3H)	
9n	7.34 (m, 5H)	8.90, 6.95	3.95, 2.65, 1.95	4.12 (q, 2H), 1.27 (t, 3H)	–
9o	7.32 (m, 5H)	8.86, 6.67	3.63, 2.63, 1.93	4.93 (h, 1H), 1.20 (d, 6H)	–
9p	7.30 (d, 2H), 6.97 (d, 2H)	8.40, 6.20	3.80, 2.37, 1.97	3.67 (s, 3H)	3.87 (s, 3H, CH_3O)
9q	7.27 (d, 2H), 6.98 (d, 2H)	8.18, 6.23	3.78, 2.37, 1.88	4.15 (q, 2H), 1.23 (t, 3H)	3.85 (s, 3H, CH_3O)
9r	7.23 (d, 2H), 6.90 (d, 2H)	8.16, 6.18	3.67, 2.32, 1.85	4.97 (h, 1H), 1.22 (d, 6H)	3.83 (s, 3H, CH_3O)
9s[a]	8.10 (d, 2H), 7.79 (d, 2H)	exch, exch	3.83, 2.70, 1.81	3.70 (s, 3H)	–
9t[a]	8.14 (d, 2H), 7.79 (d, 2H)	exch, exch	3.80, 2.70, 1.81	4.79 (h, 1H), 1.17 (s, 6H)	–
9u	6.95 (m, 3H)	8.15, 6.32	3.83, 2.35, 1.98	4.98 (h, 1H), 1.18 (d, 6H)	3.95 (2s, 6H, 2 CH_3O)
9v	6.83 (m, 3H)	8.12, 6.23	3.73, 2.40, 1.96	3.73 (s, 3H)	4.33 (m, 4H, OCH_2CH_2O)
9w	6.80 (m, 3H)	8.05, 6.42	3.80, 2.33, 1.98	5.00 (h, 1H), 1.26 (d, 6H)	4.38 (m, 4H, OCH_2CH_2O)

[a]In $CDCl_3/CD_3OD$; [b]R = CH_3, C_2H_5 or i-C_3H_7. s = singlet; d = doublet; t = triplet; q = quartet; h = septet.

Table III. Antifungal activity of **9a–w** against 36 strains of *Candida* (MIC in μg/ml).

Compound	*C albicans*	*C krusei*	*C glabrata*	*C tropicalis*	*C parapsilosis*
9b					
YNBG	58	48	64	32	32
CAS	33	32	32	17	19
9c					
YNBG	22	16	16	16	16
CAS	19	16	16	16	11
9d					
YNBG	6	6	16	16	12
CAS	5	4	16	16	8
9h					
YNBG	68	32	32	33	37
CAS	30	32	16	30	35
9i					
YNBG	101	128	64	106	68
CAS	70	128	64	110	60
9j					
YNBG	112	64	64	36	48
CAS	98	64	64	30	36
9l					
YNBG	58	32	32	36	30
CAS	47	32	32	28	30
9r					
YNBG	24	32	32	28	24
CAS	20	16	32	20	24
9s					
YNBG	10	8	8	14	18
CAS	11	8	8	16	16
9t					
YNBG	12	8	8	12	16
CAS	10	8	4	8	10
9w					
YNBG	38	64	64	24	28
CAS	36	32	32	18	16
Econazole	11	16	13	28	20

Results are not reported for **9a**, **9c**, **9e–g**, **9k** or **9m–q** whose MIC > 128 μg/ml for all strains.

Experimental protocols

Chemistry

*General method for preparation of **9a–w***

To 0.010 mol of hydrochloride of the appropriate ester in 20 ml water was added 4 ml triethylamine (0.030 mol), and then dropwise 0.010 mol of the appropriate isothiocyanate in 20 ml acetone. The mixture was stirred for 4 h at 40°C, and then evaporated under reduced pressure. The residue was suspended in water and extracted with methylene chloride. The organic layer was dried on $MgSO_4$ and concentrated under reduced pressure. The crude residue (a white-yellow oil) was triturated with diethyl ether to give a white powder, which was purified by recrystallization in THF.

*Physicochemical data for **9a–w***

Melting points were determined on a Kofler bank. Yields are given for the final step of the synthesis. Infrared data (in KBr pellets) were measured with a Perkin-Elmer 983-G spectro-

Table IV. Antifungal activity of **9a–w** against six strains of *Aspergillus* and two strains of *S brevicaulis* (MIC in μg/ml).

Compound	*A fumigatus*	*A flavus*	*A niger*	*S brevicaulis*
9b				
YNBG	32	64	64	32
CAS	16	32	16	32
9c				
YNBG	32	64	64	48
CAS	24	32	32	32
9d				
YNBG	6	8	8	8
CAS	2	4	4	4
9h				
YNBG	48	64	64	64
CAS	32	32	32	32
9i				
YNBG	64	111	64	64
CAS	64	111	64	64
9j				
YNBG	64	> 128	64	64
CAS	32	64	32	64
9l				
YNBG	64	> 128	64	64
CAS	64	> 128	32	64
9r				
YNBG	64	111	48	32
CAS	32	64	64	64
9s				
YNBG	12	16	12	8
CAS	4	8	2	4
9t				
YNBG	12	8	8	16
CAS	4	8	8	8
9w				
YNBG	48	48	64	64
CAS	32	32	64	32
Econazole	4	4	2	64

Results are not reported for **9a**, **9c**, **9e–g**, **9k** or **9m–q** whose MIC > 128 μg/ml for all strains.

meter, ν (in cm^{-1}): NH 3300–3400 and 3200–3240; CO 1710–1730. NMR data were collected in $CDCl_3$ or $CDCl_3/CD_3OD$ 4:1 for nitro derivatives which are insufficiently soluble in $CDCl_3$, at 60 MHz and with Me_4Si as an internal standard. Elemental analyses were in agreement with the accepted norms and are not reported.

Parasitology

Antifungal activity

All compounds were tested against an array of clinical isolates and one reference strain: 25 *C albicans* strains and one *C albicans* AFNOR (ATCC 2094), reference one *C glabrata*, one *C krusei*, four *C parapsilosis*, four *C tropicalis*, two *A fumigatus*, two *A niger*, two *A flavus* and two *S brevicaulis*.

MIC were performed on two media, YNBG and CAS, with solutions of **9a–w** in DMSO/water (10:90) using the method of dilution on gelose [15, 16]. On the same medium, no antifungal activity was noted for the DMSO/water mixture.

Compounds **9a–w** were screened *in vitro* against infectious larvae of an intestinal parasite of rats, *N brasiliensis*, and infectious larvae of a filaria *M dessetae*. These two tests were chosen because they tend to detect *in vitro* activities which are generally confirmed *in vivo*; experimental procedures have been published previously [12–14].

Table V. *In vitro* anthelmintic activity of **9a–w** derivatives (EC_{50} in μg/ml).

Compound	*L3 of N brasiliensis*		*L3 of M dessetae*	
	24 h	*96 h*	*24 h*	*168 h*
9a	I	I	I	20
9b	200	120	45	28
9c	200	95	48	30
9f	180	80	I	10
9g	I	I	80	30
9i	I	110	120	50
9l	I	I	I	35
9o	I	100	I	5
9p	I	I	120	3
9q	I	I	32	25
9r	150	75	50	25
9s	200	100	80	5
9u	180	125	100	50
9w	200	200	125	30
Tetramisole	1.7	0.2	65	3

I: inactive compound (EC_{50} > 200 μg/ml). Results are not reported for **9d–e**, **9h**, **9j–k**, **9m**, **9t** or **9v** whose EC_{50} > 200 μg/ml for all strains. L3 = third larval instar.

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