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Short communication

Carbamimidates, carbamimidothioates and related compounds. The effect of lipophilicity on the antibacterial activity

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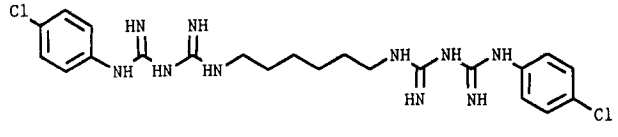
Summary — A series of carbamimidates and carbamimidothioates structurally related to chlorhexidine were prepared and tested for their antibacterial activity. The most effective compounds showed activities very similar to chlorhexidine, although, lacking in the width of the antibacterial spectrum.

Résumé — **Les carbamimidates, les carbamimidothioates et composés apparentés. Effet de la lipophilie sur l'activité antibactérienne.** *Une série de carbamimidates et de carbamimidothioates dont la structure est liée à celle de la chlohexidine fut préparée et leur activité antibactérienne testée. Les composés les plus efficaces révélèrent des activités semblables à celles de la chlorhexidine mais moins larges dans le spectre antibactérien.*

bactericide / synthesis / carbamimidates / carbamimidothioates / lipophilicity / structure–activity relationships

The bactericidal efficacy of biguanides and their bis-derivatives was revealed in the 1940's [1, 2] in connection with work that eventually led to the development of proguanil as an antimalarial agent. Biguanides with hypoglycaemic [3] and antiviral [4, 5] activities are also known, but the flagship of this class of compounds is undoubtedly chlorhexidine (**1**) (Hibitane) [6–8]. Chlorhexidine exhibits a broad antibacterial spectrum and is non-toxic toward mammalian cells. The most profound lack of activity is that against Gram-negative bacteria, especially against *Pseudomonas aeruginosa*. Chlorhexidine, as well as a number of other related compounds, has shown promising preventive activity against *Streptococcus*-initiated dental plaque formation [9].



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The antibacterial activity of biguanides was initially believed to require two biguanide moieties. However, in a series of research incorporating carbamimidates, it was shown that a single biguanide unit or its bioisostere is sufficient to induce the desired activity [4, 5].

The mode of action of chlorhexidine is not clear at present. It is known that at low concentrations it inhibits bacterial cell membrane bound ATPase, at higher concentrations it induces leakage of cytoplasmic components, and at concentrations above 100 µg/ml it precipitates cytoplasmic proteins [9].

In order to gain more insight into the effects of bioisosteric replacements of the nitrogen functions, we set out to examine the effects of overall lipophilicity on the antibacterial activity of aminoiminomethylthioureas, -imidates and -thioimidates(*). A number of compounds were prepared and tested against representative Gram(+) and Gram(–) bacteria and yeast cells. We report the salient features of our findings.

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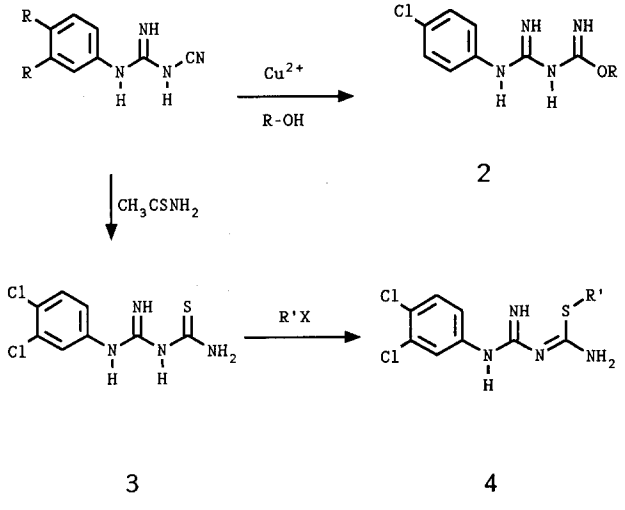
(*)The nomenclature of these types of compounds is quite confusing: amidinourea, carbamidinourea, guanylurea and (aminoiminomethyl) urea are used interchangeably. Synonyms used for amidinoimidates include carbamidinoisoureas and amidinocarbamimidates, not to mention the Chemical Abstracts azaalkane and dicarbonic acid nomenclatures.

Chemistry

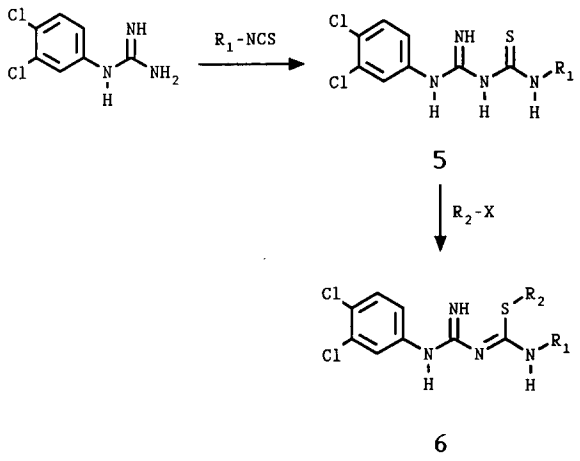
The aminoiminomethyl-carbamimidates **2** (R = alkyl or alkoxyalkyl) were prepared from the corresponding *N*-(4-chlorophenyl)-*N'*-cyanoguanidine through Cu^{2+} catalyzed addition of the appropriate alcohol [5]. Butyl and octyl residues were chosen to represent the more lipophilic compounds, whereas 2-methoxyethyl and 2-(2-methoxyethoxy)ethyl derivatives represent the more hydrophilic ones.

For the preparation of the derivatives containing the shorter alkyl chains (**2a**, **b**), the corresponding alcohol was used as the reaction solvent, whereas the longer chains (**2c**, **d**) were introduced using dimethyl cellosolve as the solvent, and employing an excess of the respective alcohol. The S-alkylated aminoiminomethyl-carbamimidothioates **4** were prepared in a straightforward manner by reacting the aminoiminomethyl-thio-ureas **3** [10] with the corresponding alkyl halides (scheme 1).

N-Substituted aminoiminomethylthioureas **5** and aminoiminomethyl-carbamthioates **6** were prepared from 3,4-dichlorophenyl-guanidine (scheme 2). Its reaction with various isothiocyanates led to the thioureas **5**. These could be conveniently purified by conversion to the highly crystalline maleate salts. After liberation of the free base, the compounds **5** were reacted with alkyl halides to provide the *N*,*S*-disubstituted carbamimidothioates **6**. With alkyl bromides the reaction temperature had to be elevated to ca 80°C. If both alkyl chains were bulky, the reactions tended to become considerably slower. Prolonged reaction time and especially activation of the alkyl halide leaving group by KI catalysis were needed to ensure complete conversion. The products were purified by crystallization from ethyl acetate.



Scheme 1.



Scheme 2.

The physical data for compounds **4–6** are presented in table I. The lipophilicities were estimated using the simple additive rules described by Hansch *et al* [11] and the published substituent constants [12]. These values are shown in table II and III in the form of fragment constants (π values) for the varying substituents.

Microbiology

The minimum inhibitory concentrations (MIC) were determined against *Staphylococcus aureus, Escherichia coli, Proteus vulgaris* [Gram(+)], *Pseudomonas aeruginosa* [Gram(–)] and *Candida albicans* [fungus]. The results are shown in table II, along with those obtained for chlorhexidine under the same conditions. *Pseudomonas* and *Proteus* remained resistant to these compounds, with the exception of carbamimidates **2a** and **2b** where slight activity was seen.

From the MIC data on compounds **2a–d** it is clear that changing the lipophilic butyl and octyl chains (**2a**, **c**) to the polyethers (**2b**, **d**) abolishes the activity. Replacement of a methylene by an ether oxygen is expected to decrease the lipophilicity by approximately 2 log P-units [12, 13]. Compounds **2b** and **2d** have (calculated) log P values considerably lower than compounds **2a** and **2c**. Although the lipophilicity may not be the only factor affecting antibacterial efficacy, the qualitative agreement with the observations is good.

The MIC-values for compounds **4–6** against *S aureus*, *E coli* and *C albicans* are shown in table III. The parent compound **3** is completely devoid of any activity apart from the very weak inhibition of *S aureus*.

Table I. Physical and spectral data of compounds **2**, **4**, **5** and **6**.

Cmpd (salt)	$R(R_1)$	R_2	mp. [°C]	spectral data
2a (HOAc)	n-C_4H_9	-	158-60	(lit.; 158-59[4]) NMR: 0.9-1.65 (m, 7H), 1.80 (s, 3H), 4.11 (t, 2H), 6.95-7.45 (m, 4H), 8.1 (br) IR: 1660, 1592, 1500, 1346 cm^{-1} $C_{12}H_{17}N_4OCl.C_2H_4O_2$ calc: C 51.1, H 6.4, N 17.0 found: C 51.5, H 6.5, N 16.8
2b (HOAc)	$(CH_2)_2OMe$-		146-7	NMR: 1.84 (s, 3H), 3.26 (s, 3H), 3.55-4.10 (AA'BB', 4H), 6.93-7.38 (m, 4H), 7.95 (br) $C_{11}H_{15}N_4O_2Cl.C_2H_4O_2$ calc: C 47.2, H 5.7, N 16.9 found: C 47.4, H 6.0, N 16.7
2c (HOAc)	n-C_8H_{17}	-	143-44	(lit.: 145-6[4])
2d (HOAc)	$(CH_2)_2O(CH_2)_2OMe$		122	NMR (200): 1.79 (s, 3H), 3.24 (s, 3H), 3.41-3.55 (AA'BB', 4H),7.5-7.32 (m, 4H), 8.1 (br) $C_{13}H_{19}N_4O_3Cl.C_2H_4O_2$ calc: C 48.1, H 6.1, N 14.9 found: C 47.8, H 6.4, N 14.7
4a (HI)	CH_3	-	150	NMR: 2.43 (s, 3H), 7.15- 7.7 (m, 3H), 8.23, 8.52, 10,55 (3x br s) $C_9H_{10}N_4SCl_2.HI$ calc: C 26.7, H 2.7, N 13.8 found: C 26.2, H 2.6, N 13.6
4b (HBr)	n-C_4H_9	-	156-8	NMR: 0.8-1.6 (m, 7H), 2.91 (s, 3H), 7.15-7.72 (m, 3H), 8.23, 8.59, 10.59 (3x br s) IR: 1642, 1522, 1380 cm^{-1} $C_{12}H_{16}N_4SCl_2.HBr$ calc: C 36.0, H 4.2, N 14.0 found: C 36.3, H 4.4, N 13.8
4c (HBr)	n-C_8H_{17}	-	151-3	NMR (200): 0.84 (t, 3H), 1.19 (m, 12H), 2.87 (t, 2H), 7.20-7.69 (m, 3H), 8.23, 8.50, 10.39 (3x br s) IR: 1642, 1511, 1128 cm^{-1} $C_{16}H_{24}N_4OCl_2.HBr$ calc: C 42.1, H 5.5, N 12.3 found: C 42.0, H 5.6, N 12.4
4d (HBr)	n-$C_{12}H_{25}$	-	130-1	NMR: 0.84 (t, 3H), 1.2-1.55 (m, 20H), 2.87 (t, 2H), 7.1-7.7 (m, 3H), 8.23, 8.56, 10.83 (3x br s) IR: 2950, 1660, 1552, 1390 cm^{-1} $C_{20}H_{33}N_4SCl_2.HBr$ calc: C 46.9, H 6.4, N 10.9 found: C 46.6, H 6.4, N 11.1
5a (maleate)	C_2H_5	-	180-1	NMR (200): 1.12 (t, 3H), 3.24, 3.48 (dq, 2H), 6.26 (m, 2H), 7.21-7.98 (m, 3H), 7.1-9.2 (5x br) $C_{10}H_{12}N_4SCl_2.C_4H_4O_4$ calc: C 41.3, H 3.9, S 7.9 found: C 40.9, H 4.0, S 7.8
5b (maleate)	n-C_4H_9	-	149	NMR (200): 0.87 (m, 3H), 1.30 (m, 2H), 1.52 (m, 2H), 2.35 (m, 2H), 3.20, 3.47 (dq, 2H), 6.24 (s, 2H), 7.35-7.95 (m, 3H), 8.3-10.0 (3x br s) IR: 1692, 1618, 1560, 1482 cm^{-1} $C_{12}H_{16}N_4SCl_2.C_4H_4O_4$ calc: C 44.1, H 4.6, S 7.4 found: C 43.8, H 4.5, S 7.3
5c (maleate)	n-C_8H_{17}	-	146	NMR (200): 0.8-1.52 (m, 15H), 3.19, 3.47 (dq, 2H), 6.25 (s, 2H), 7.2-7.93 (m, 3H), 7.0-9.2 (4 x br) $C_{16}H_{24}N_4SCl_2.C_4H_4O_4$ calc: C 48.9, H 5.7, S 6.5 found: C 48.7, H 5.7, S 6.5
5d (maleate)	n-$C_{12}H_{25}$	-	140-2	NMR: 0.8-1.25 (m, 23H), 2.7 (m, 2H), 6.27 (s, 2H), 7.1-7.9 (m, 3H), 8-10.5 (3x br s) $C_{20}H_{32}N_4SCl_2.C_4H_4O_4$ calc: C 52.7, H 6.6, S 5.9 found: C 52.3, H 6.7, S 5.8
6a (HI)	C_2H_5	CH_3	132-5	NMR: 1.13 (t, 3H), 2.46 (s, 3H), 3.33 (q, 2H), 7.17-7.72 (m, 3H), 8.06, 8.95, 10.16 (3x br s) IR: 1640, 1569, 1532, 1482 cm^{-1} $C_{11}H_{14}N_4SCl_2.HI$ calc: C 30.5, H 3.5, N 12.9 found: C 30.7, H 3.5, N 13.1
6b (HBr)	C_2H_5	n-C_4H_9	179	NMR (200): 0.81 (t, 3H), 1.14 (t, 3H), 1.28 (m, 2H), 1.46 (m, 2H), 2.95 (t,2H), 3.32 (q, 2H), 7.23-7.69 (m, 3H), 8.15, 9.11, 10.4 (3x br s) IR: 1659, 1585, 1542, 1492 cm^{-1} $C_{14}H_{20}N_4SCl_2.HBr$ calc: C 39.3, H 4.9, N 13.1 found: C 39.5, H 4.8, N 12.7
6c (HBr)	C_2H_5	n-C_8H_{17}	99-101	NMR (200): 0.85-1.49 (m, 19H), 2.87 (t, 2H), 3.18 (q, 2H), 7.24-7.69 (m, 3H), 8.22, 9.9, 10.5 (3x br s) $C_{18}H_{28}N_4SCl_2.HBr$ calc: C 44.6, H 6.0, N 11.6 found: C 44.8, H 6.1, N 11.7
6d (HBr)	n-C_4H_9	n-C_4H_9	129-30	IR: 1645, 1576, 1529, 1480 cm^{-1} $C_{16}H_{24}N_4SCl_2.HBr$ calc: C 42.1, H 5.5, N 12.3 found: C 42.5, H 5.5, N 12.4
6e (HBr)	n-C_4H_9	n-C_8H_{17}	113-15	IR: 1660, 1589, 1542, 1496 cm^{-1} $C_{20}H_{32}N_4SCl_2.HBr$ calc: C 46.9, H 6.5, N 10.9 found: C 47.3, H 6.5, N 10.8
6f (HBr)	n-C_4H_9	n-$C_{12}H_{25}$	108-10	IR: 1660, 1578, 1545, 1494 cm^{-1} $C_{24}H_{40}N_4SCl_2.HBr.\frac{1}{2}H_2O$ calc: C 49.9, H 7.3, N 9.7 found: C 49.9, H 7.3, N 10.0
6g (HI)	n-C_8H_{17}	CH_3	102-3	NMR (200): 0.85 (t, 3H), 1.21 (br s, 10H), 1.48 (m, 2H), 2.48 (s, 3H), 3.23 (t, 2H), 7.22-7.67 (m, 3H), 8.06, 8.97, 10.2 (3x br s) IR: 1645, 1574, 1530, 1485 cm^{-1} $C_{17}H_{26}N_4SCl_2.HI$ calc: C 39.5, H 5.3, N 10.8 found: C 39.3, H 5.3, N 10.4
6h (HBr)	n-C_8H_{17}	n-C_4H_9	110-11	IR: 1660, 1583, 1545, 1490 cm^{-1} $C_{20}H_{32}N_4SCl_2.HBr$ calc: C 46.9, H 6.5, N 10.9 found: C 46.6, H 6.6, N 11.0
6i (HBr)	n-C_8H_{17}	n-C_8H_{17}	111-12	IR: 1660, 1583, 1544, 1490 cm^{-1} $C_{24}H_{40}N_4SCl_2.HBr$ calc: C 50.7, H 7.2, N 9.9 found: C 51.1, H 7.1, N 9.7
6j (HBr)	n-C_8H_{17}	n-$C_{12}H_{25}$	101	IR: 1659, 1579, 1540, 1487 cm^{-1} $C_{28}H_{48}N_4SCl_2.HBr$ calc: C 53.8, H 7.9, N 9.0 found: C 54.1, H 7.8, N 8.8
6k (HBr)	n-$C_{12}H_{25}$	n-C_8H_{17}	87-8	IR: 1658, 1578, 1540, 1482 cm^{-1} $C_{28}H_{48}N_4SCl_2.HBr$ calc: C 53.8, H 7.9, N 9.0 found: C 54.2, H 7.9, N 9.1

Table II. Minimum inhibitory concentrations (μg/ml) and calculated lipophilicities (π values) for carbamimidates **2**.

	R	π	*Staph. aureus*	*Pseud. aeruginosa*	*E. coli*	*Prot. vulgaris*	*Candida albicans*
1			2	16	8	32	8
2a	n-C_4H_9	2.1	63	250	63	125	125
2b	$(CH_2)_2OMe$	-0.1	>500	>500	>500	>500	>500
2c	n-C_8H_{17}	4.1	8	125	8	125	16
2d	$(CH_2)_2O(CH_2)_2OMe$	0.1	500	>500	>500	>500	>500

Table III. Minimum inhibitory concentrations (μg/ml) and calculated lipophilicities (π values for SR and NR) for amidinothioureas **3**, **5** and carbamimidothioates **4**, **6**.

	-SR	-NR	π	*Staph. aureus*	*E. coli*	*Candida albicans*
3	-	H		32	>500	>500
4a	CH_3	H	0.6	32	125	63
4b	C_4H_9	H	2.1	32	16	32
4c	C_8H_{17}	H	4.1	8	16	16
4d	$C_{12}H_{25}$	H	6.1	125	>500	125
5a	-	C_2H_5	1.0	32	125	125
5b	-	C_4H_9	2.1	125	>500	500
5c	-	C_8H_{17}	4.1	>500	>500	>500
5d	-	$C_{12}H_{25}$	6.1	>500	>500	>500
6a	CH_3	C_2H_5	1.6	63	500	125
6b	C_4H_9	C_2H_5	3.1	16	32	32
6c	C_8H_{17}	C_2H_5	5.1	8	32	32
6d	C_4H_9	C_4H_9	4.1	8	63	250
6e	C_4H_9	C_8H_{17}	6.2	16	250	32
6f	C_4H_9	$C_{12}H_{25}$	8.2	>500	>500	>500
6g	C_8H_{17}	CH_3	4.7	16	32	32
6h	C_8H_{17}	C_4H_9	6.2	250	>500	125
6i	C_8H_{17}	C_8H_{17}	8.2	32	125	32
6j	C_8H_{17}	$C_{12}H_{25}$	10.2	250	>500	250
6k	$C_{12}H_{25}$	$C_{12}H_{25}$	12.2	500	>500	>500

Thioureas **5** and carbamimidothioates **4** exhibit an interesting complementary behavior. The weak activity of thioureas **5** diminishes on lengthening the *N*-alkyl chain, eventually leading to no detectable activity with an octyl chain. With carbamimidothioates **4**, the *S*-octyl derivative exhibits the lowest MIC-values against all three test organisms in this series. As can be seen in the *N,S*-disubstituted series **6** the lipophilic character cannot be over-emphasized. Complete loss of measurable antibacterial activity if either of these substituents is too long (C_{12}) is most obvious. This may be due to micelle formation or related phenomena leading to unfavorable distribution of the compounds.

The results against *Staphylococcus aureus* are shown in figure 1. Maximal activity in these compounds is found when the sum of the alkyl chain carbon atoms is 8–10.

Furthermore, it is interesting to note that *S*-alkylation is nearly always necessary for good activity. It is evident from the data in table III that the lack of *S*-substitution diminishes (or even abolishes) the antibacterial activity. Compound **4c** with an octyl *N*-substituent, is the most active member of the series, closely followed by *eg* **6c** and **6d**. This notion suggests that the optimal activity is reached in cases where the sum of the *N,S*-substituent carbon atoms is eight, and both the *N* and *S* are substituted.

Conclusions

A number of arylaminoiminomethylthiourea derivatives substituted at the terminal nitrogen and sulfur atoms have been tested for their antibacterial activities. Substituted thiourea derivatives **5** exhibit the lowest activities. Sulfur substitution enhances the activity. Generally, the substitution pattern has little effect on the activity, as long as the correct overall lipophilicity is maintained. In the case of carbamimidothioates **4** and **6**, the optimal lipophilicity is obtained when the sum of the carbon atoms in the substituents represents an octyl chain.

The presence of sulfur in place of oxygen has little effect, as can be seen by comparing the MIC values of compounds **2c** and **4c**. Finally, the most effective compounds, although functionally rather different from chlorhexidine **1**, show activities very similar to **1**, although, lacking in the width of the antibacterial spectrum.

Experimental protocols

Melting points were determined on a Koffler hot-plate apparatus and are uncorrected. The NMR spectra were measured on a JEOL JNM-PMX 60 (60 MHz) or a Bruker AC-200 spectrometer in $CDCl_3$ (TMS as internal standard, δ = 0.00 ppm). The IR spectra were measured with a Hitachi 270-30 instrument.

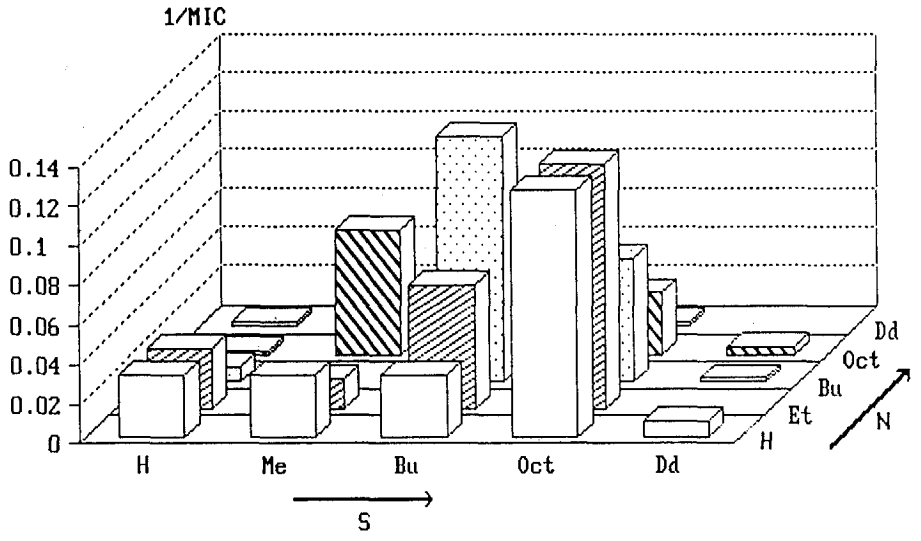


Fig 1. Graphical presentation of the activities of compounds **3–6** against *Staphylococcus aureus.*

Alkyl N-*[(4-chlorophenylamino)iminomethyl]carbamimidates* ***2a–d***

General procedure

These compounds were prepared according to Warner *et al* [4] with the following modifications. Instead of using gaseous H_2S, 2 mol equivalents of NaSH were used to remove Cu^{2+}. In the case of **2a, b** the neat alcohol was used as the reaction medium. For **2c, d** a mixture of dimethyl cellosolve and 6 mol equivalents of the appropriate alcohol was used. At the end of the work-up the mother liquors of **2a, b** and **d** were saturated with KOAc to initiate crystallization of the products as their acetate salts.

N'-*[(arylamino)iminomethyl]*-N-*alkyl thioureas* ***5a–d*** were prepared according to the following representative example:
N'*[(3,4-dichlorophenylamino)iminomethyl]*-N-*ethyl thiourea maleate* ***5a***. A solution of 3,4-dichlorophenylguanidine (3.8 g, 18.6 mmol) and ethyl isothiocyanate (1.6 g, 18.4 mmol) in 100 ml acetonitrile was refluxed overnight. The mixture was evaporated to dryness under vacuum and the residue was dissolved in 30 ml ethanol. Maleic acid (2.1 g, 18.4 mmol) was added, and the product was collected by filtration. Yield: 5 g, 67%. mp 174°C.

The free base was liberated by suspending the salt in methanol, and neutralizing the solution with aq ammonia. The clear solution was diluted with water and the free base was extracted into chloroform.

Alkyl N-*[(arylamino)iminomethyl]carbamimidothioates* ***4a–d*** were prepared according to the following representative example:
Octyl N-*[(3,4-dichlorophenylamino)iminomethyl]-carbamimidothioate* ***4c***. A mixture of 3,4-dichlorophenylamino-iminomethylthiourea (0.5 g, 1.9 mmol) and 1-bromo-octane (0.36 ml, 0.4 g, 2.1 mmol) in 5 ml isopropanol was refluxed overnight. After cooling to room temperature, the mixture was filtered and evaporated. The waxy residue was dissolved into 0.5 ml acetone, and hexane (2 ml) was added to induce crystallization, yielding 0.69 g (97%) of **4c** mp 142–146°C.

Alkyl N-*[(3,4-dichlorophenylamino)-iminomethyl]*-N'-*alkylcarbamimidothioates* ***6a–k*** were prepared according to the following general example:
Methyl-N-*[(3,4-dichlorophenylamino)iminomethyl]*-N'-*octylcarbamimidothioate* ***6g***. A mixture of thiourea **5c** free base (2.06 g, 5.5 mmol) and methyl iodide (0.34 ml, 5.5 mmol) in 20 ml isopropanol was stirred overnight at 40°C. The solution was evaporated to dryness, and the oily residue was dissolved in 5 ml ether. Hexane (50 ml) was added slowly and the solution was placed in a refrigerator to induce crystallization of the product. Yield: 1.75 g (82%).

Microbiology

Minimum inhibitory concentrations (MIC) were determined according to previously described methods [14], using the following micro-organisms: *Pseudomonas aeruginosa* NCTC 6749, *Proteus vulgaris* NCTC 4635, *Staphylococcus aureus* NCTC 4163, and *Escherichia coli* NCTC 3196. The concentrations were varied between 500 μg/ml to 1 μg/ml, using serial dilution. The tests were performed on microtiter plates, and the bacterial growth was measured by turbidometry after incubation (37°C, 18 h). The results are shown in tables II and III.

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