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Triorganotin compounds as antimicrobial agents

Federica Novelli [a], Marco Recine [a], Fabio Sparatore [a,*], Claudia Juliano [b]

[a] *Dipartimento di Scienze Farmaceutiche, Università di Genova, Viale Benedetto XV, I-16132 Genoa, Italy*
[b] *Dipartimento di Scienze del Farmaco, Università di Sassari, Via Muroni 23a, I-07100 Sassari, Italy*

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Abstract

Six triorganotin derivatives of thiolupinine(1-mercaptolupinane), 2-mercaptobenzoxazole and 2-mercaptobenzothiazole were prepared and tested against several bacteria, fungi and protozoa. Most compounds exhibited high activity against the tested microorganisms and particularly worth noting was the activity of triethyltin lupinylsulfide on Gram-negative strains. Triethylgermanium lupinylsulfide was also prepared but was devoid of action on the whole set of tested microorganims.

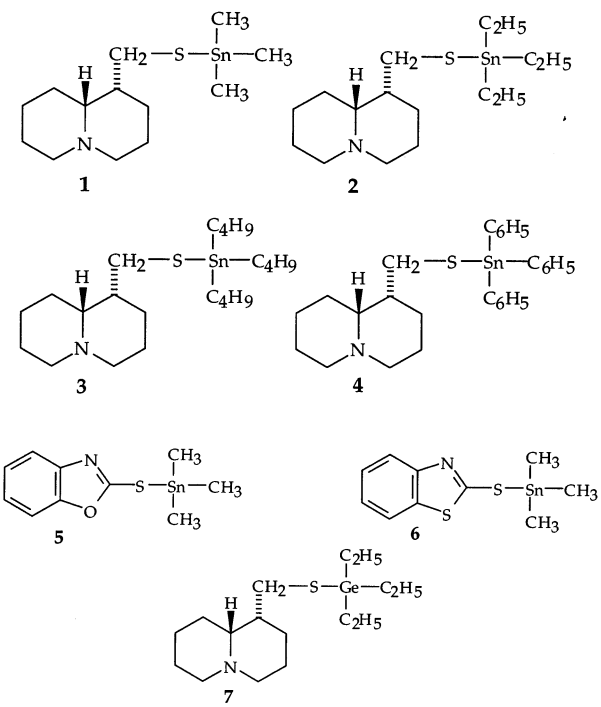
Keywords: Organotin compounds; Thiolupinine derivatives; 2-Mercaptobenzazole derivatives; Antimicrobial agents

1. Introduction

The growing interest in metal-based chemotherapy of neoplastic, parasitic and infectious diseases, prompted us to undertake the study of antitumor and antimicrobial activity of some gold and tin derivatives of tertiary aminothiols. Among these, thiolupinine—which was recently prepared by us [1]—is peculiar for its high liposolubility and strong basicity, which should warrant the capability of its derivatives to pass through biological barriers and also produce water soluble salts.

In a previous paper [2] we described the activity (from moderate to very high) of seven gold complexes against some strains of *Escherichia coli*, *Staphylococcus aureus*, *S. epidermidis*, *Candida albicans*, *Aspergillus niger* and *Trichomonas vaginalis*; the same compounds were devoid of activity (MIC ≥ 250 μg/ml) on *Pseudomonas aeruginosa*.

Here we describe the activity of six triorganotin derivatives of thiolupinine, 2-mercaptobenzoxazole and 2-mercaptobenzothiazole against the above microorganisms; additionally the activity of triethylgermanium lupinylsulfide was explored. According to Sijpeisteijn et al. [3], organogermanium compounds should be endowed with antimicrobial activity similarly to organotins.



The biocidal activity of organotin compounds was recognized in the mid-1950s and presently organotin compounds represent important industrial and agricultural biocides, wood preservatives and marine antifoulants [4].

* Corresponding author. Fax +39-010-3538358.
E-mail address: sparator@unige.it (F. Sparatore)

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Trimethyltins are highly insecticidal, while tripropyl-, tributyl- and triphenyltin compounds have a high degree of fungicide and bactericide activity, which is most effective against Gram-positive bacteria.

Some triorganotin complexes of Schiff bases are potently active against *Entamoeba histolytica*: one compound is much more active than emetine [5].

Triorganotin compounds have also been used to control the infective vector of *Schistosoma* and mosquitoes [6].

Dialkyltin compounds are less active than the corresponding trialkyltins as antimicrobial agents, but are also substantially less toxic.

Various dialkyltin compounds exhibit antihelmintic properties and are widely used for veterinary (particularly poultry) helminthiasis and coccidiosis [7]. Dioctyltin maleate is amongst the most active compounds employed in the control of the *Leishmania* species in mice [8].

Dibutyltin derivatives of 2-mercaptobenzoxazole and 2-mercaptobenzothiazole exhibit a very high activity in vitro against *Trypanosoma equiperdum*, as it has been shown quite recently [9]; unfortunately, these compounds were completely inactive in vivo.

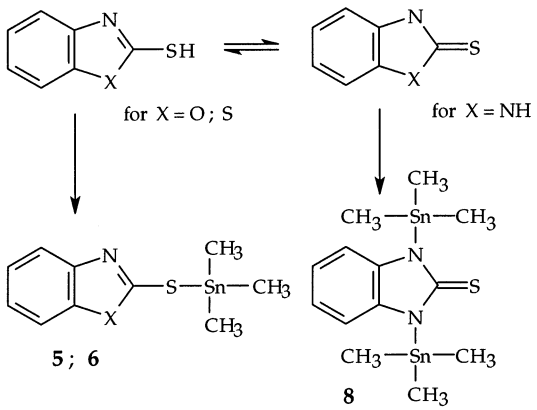
Finally, more than 2000 organotin compounds were tested by the NCI for anticancer activity and many of them ($\sim$ 29%, mainly diorganotins) demonstrate activity against murine P388 leukemia (but not against L1210 leukemia) and some of these show reproducible antitumor activity in mice and rats. Although this activity is lower than that of cisplatin, the toxic side effects of platinum compounds are not seen [10,11]. Organotin compounds more active than cisplatin have been described recently: dimethyltin carboxylates were highly active against HeLa, Hep-2, RD, L_{20B} and P388 cell lines [12], while triethyltin lupinylsulfide **2** exhibit an IC_{50} around 10^{-7} M against several cell lines of multiresistant ovarian carcinomas [13].

Thus, in spite of the huge number of tri- and diorganotins investigated so far, we deemed it interesting to explore the influence on antimicrobial activity of peculiar groups (f.i., aminothiols) saturating the residual valences of the metal.

Indeed, the nature of the thiols used could prove of great importance in regulating chemical stability, in vivo absorption, distribution and topical and/or systemic toxicity of the studied compounds, eventually making them suitable for human use.

2. Chemistry

Compounds **1**–**4** and **7** were easily prepared by reacting thiolupinine with triorganotin halides and triethylgermanium chloride, respectively. Preparation of compounds **5** and **6** required the action of trimethyltin chloride on 2-mercaptobenzoxazole and 2-mercaptobenzothiazole in the presence of sodium ethoxide. The analogous reaction with 2-mercaptobenzimidazole gave the 1,3-bis(trimethyltin)benzimidazol-2-thione (**8**). Compound **8** was rather unstable in the presence of water; thus, its antimicrobial activity was not investigated.



The structures of the prepared compounds were supported by elemental analyses and spectroscopic data.

Particularly, the *S*-substitution of compounds **5** and **6** and *N*,*N*-disubstitution of compound **8** were supported by the pattern of ^{1}H NMR of aromatic protons and also by the different stability toward water. While compound **8** was rapidly hydrolyzed generating 2-mercaptobenzimidazole, compounds **5** and **6** were relatively stable.

3. Experimental

Melting points were determined by the capillary method on a Büchi apparatus and are uncorrected.

Elemental analyses were performed at the Microanalytical Laboratory of the 'Dipartimento di Scienze Farmaceutiche' of Genoa University and the analytical results for C, H, N and S were within $\pm$0.3% of the calculated values, unless otherwise stated.

UV spectra were recorded with a Perkin–Elmer 550S spectrophotometer; ^{1}H NMR spectra were taken on a Varian Gemini 200 spectrometer, using $CDCl_3$ or DMSO-d_6 as solvent.

*3.1. Trimethyltin lupinylsulfide hydrochloride (**1**) and triethyltin lupinylsulfide hydrochloride (**2**)*

Trimethyltin chloride (1 g, 5 mmol) or triethyltin bromide (1 g, 3.5 mmol) and freshly distilled stoichiometric thiolupinine were dissolved in peroxide-free THF ($\sim$ 8 ml); the mixture was stirred in a closed tube for 24 h at room temperature (r.t.). The solvent was removed under reduced pressure and the oily residue

was dissolved in water, treated with 6 N NaOH and extracted with ether. The ether extract residue was chromatographed on neutral alumina (1:20) eluting with dry ether: 1.12 g of trimethyltin lupinylsulfide (64.1% yield) or 1.16 g of triethyltin lupinylsulfide (85% yield) were obtained as oily, colorless, free bases.

Analysis (C, H, N, S) for trimethyltin lupinylsulfide according with $C_{13}H_{27}NSSn$, but +0.6% on carbon and nitrogen were found.

Analysis (C, H, N, S) for triethyltin lupinylsulfide according with $C_{16}H_{33}NSSn$, but +0.4% on nitrogen was found.

The free bases were converted to the hydrochlorides by dissolving them in the stoichiometric volume of 0.1 N ethanolic HCl and removing the solvent under reduced pressure.

Analysis (C, H, N, S) for compound **1** according with $C_{13}H_{27}NSSn \cdot HCl \cdot 0.5H_2O$, but +0.6% on nitrogen and −0.5% on sulfur were found.

Analysis (C, H, N, S) for compound **2** according to $C_{16}H_{33}NSSn \cdot HCl$.

3.2. Tributyltin lupinylsulfide hydrogen fumarate (3)

Tributyltin chloride (1 g, 3 mmol) and freshly distilled thiolupinine (0.57 g, 3 mmol) were dissolved in methanol (~8 ml); the mixture was stirred in a closed tube for 4 h at r.t. The solvent was removed under reduced pressure; the oily residue was dissolved in absolute ethanol, basified with 10% NH_3, filtered and the solution was evaporated to dryness. The residue was chromatographed on neutral alumina (1:30) eluting with dry ether: 0.95 g (yield 65.2%) was obtained as an oily, colorless, free base.

Analysis (C, H, N, S) for tributyltin lupinylsulfide according to $C_{22}H_{45}NSSn$, but −0.8% on carbon and −0.6% on sulfur were found.

The free base was converted to the hydrogen fumarate by dissolving it in the stoichiometric volume of 0.2 M ethanolic fumaric acid and removing the solvent under reduced pressure.

Analysis (C, H, N, S) for compound **3** according with $C_{22}H_{45}NSSn \cdot C_4H_4O_4$, but −0.4% on carbon and sulfur, +0.5% on hydrogen and +0.6% on nitrogen were found.

3.3. Triphenyltin lupinylsulfide hydrochloride (4)

Triphenyltin chloride (1.5 g, 3.7 mmol; 95% purity) and freshly distilled thiolupinine (0.68 g, 3.7 mmol) were dissolved in peroxide-free THF (~8 ml); the mixture was stirred in a closed tube for 5 h at r.t. The solvent was removed, the residue was taken up several times with dry ether and dried on KOH under vacuum: 1.2 g (yield 57.3%) of a white, hygroscopic solid were obtained.

Analysis (C, H, N, S) for $C_{28}H_{33}NSSn \cdot HCl \cdot 0.5H_2O$, but −0.4% on hydrogen and nitrogen and −1.4% on sulfur was found.

3.4. 2-[(Trimethyltin)mercapto]benzoxazole (5) and 2-[(trimethyltin)mercapto]benzothiazole (6)

To 0.115 g (5 mmol) of sodium dissolved in 4 ml of absolute ethanol stoichiometric 2-mercaptobenzothiazole or 2-mercaptobenzoxazole and, after solubilization, 1 g (5 mmol) of trimethyltin chloride in 4 ml of absolute ethanol was added; the mixture was stirred in a closed tube for 4 h at r.t. The reaction mixture was filtered and the solution was evaporated to dryness: 1.415 g (yield 90.1%) of a white solid, m.p. 112–113°C.

Analysis (C, H, N, S) for $C_{10}H_{13}NOSSn$.

Compound **6**: 1.32 g (yield 80.0%) of a yellow colored oil, which separated a small amount of white solid on standing.

Analysis for $C_{10}H_{13}NS_2Sn$, but +0.7% on carbon and sulfur and +0.5% on nitrogen were found.

3.5. Triethylgermanium lupinylsulfide hydrochloride (7)

Triethylgermanium chloride (1 g, 5.1 mmol) and freshly distilled thiolupinine (0.95 g, 5.1 mmol) were dissolved in peroxide-free THF (~8 ml); the mixture was stirred in a closed tube for 22 h at r.t. The reaction mixture was filtered and the solution was evaporated to dryness; the residue was washed several times with dry ether to give 1.15 g (yield 77.3%) of white solid, m.p. 91–93°C.

Analysis for $C_{16}H_{33}NSGe \cdot HCl$.

3.6. 1,3-bis(Trimethyltin)benzimidazol-2-thione (8)

To 0.115 g (5 mmol) of sodium dissolved in 4 ml of absolute ethanol, 0.750 g (5 mmol) of 2-mercaptobenzimidazole (non-completely soluble) and 1 g (5 mmol) of trimethyltin chloride in 4 ml of absolute ethanol were added; the mixture was stirred in a closed tube for 2 h at r.t. The reaction mixture was filtered and the solution evaporated to dryness. The residue was washed several times with petroleum ether leaving the unreacted 2-mercaptobenzimidazole; after evaporation of petroleum ether the residue was treated with dry ether. The ether solution was filtered and evaporated yielding 0.338 g (14.2%) of waxy solid, releasing a gas that was irritating to mucosae.

Analysis for $C_{13}H_{22}N_2SSn_2 \cdot H_2O$, but −0.5% on carbon was found.

4. Antimicrobial activities

Compounds **1**–**7**, together with commercial triphenyltin chloride and some reference drugs, were

Table 1
MIC (μg/ml) of compounds **1**–**7** and some reference drugs

Compound	*E. coli* (ATCC25922)	*P. aeruginosa* (ATCC27853)	*S. aureus* (ATCC25923)	*S. aureus* (ATCC6538)	*S. epidermidis* (clinically isolated)	*C. albicans* (ATCC10231)	*A. niger* (ATCC16404)	*T. vaginalis* OZ-2 (clinically isolated)
1	62.5	125	250	250	125	>250	>250	62.5
2	1.95	31.25	15.63	7.81	7.81	15.63	7.81	7.81
3	>250	>250	0.49	0.122	0.49	1.95	0.98	0.98
4	125	>250	0.244	0.061	0.244	1.95	7.81	0.49
5	62.5	62.5	250	250	250	31.25	62.5	31.25
6	62.5	125	62.5	125	62.5	125	62.5	15.63
7	>250	>250	>250	>250	>250	>250	250	250
Triphenyltin chloride	125	>250	0.122	0.015	0.122			
Piperacillin	0.98	1.95	0.49	0.244	0.244			
Chloramphenicol	3.90	>250	3.90	3.90	1.95			
Miconazole						15.6	7.8	
Metronidazole								0.244

tested for their activity against the following strains of bacteria, fungi and protozoa: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 37853, *S. aureus* ATCC 25923 and ATCC 6538, *S. epidermidis* clinical isolated, *C. albicans* ATCC 10231, *A. niger* ATCC 16404, and clinically isolated *T. vaginalis* OZ-2.

The procedures for the antibacterial, antifungal and antitrichomonal assays have already been described [2].

5. Results and discussion

The results of the antimicrobial screening of compounds **1**–**7**, triphenyltin chloride and some reference drugs are shown in Table 1.

The synthesized organotin derivatives **2**–**6**, as well as the triphenyltin chloride, were rather strongly active against the considered strains of bacteria, fungi and protozoa. Compounds **2** and **5** also exhibited activity against *P. aeruginosa* (MIC = 31 and 62 μg/ml, respectively). Compound **1** was only moderately active and limited to a few strains.

The triethylgermanium compound **7**, in spite of the structural similarity with the tin compound **2**, was completely devoid of action on the whole set of microorganisms. Triethylgermanium acetate was found to be active on all of the tested strains of fungi [3].

The observed activities of triorganotin compounds were clearly related to the nature of the organic moieties bound to the metal.

The activity of compounds **1**–**4** (all derived from thiolupinine) on Gram-positive bacteria, fungi and trichomonas increased greatly with increasing organic moiety size. However, this observation does not hold for the Gram-negative bacteria, whose susceptibility decreased with increasing molecular size of the tested agent. The strong activity of the triethyltin compound **2** on Gram-negative strains was very peculiar and was superior to that of both lower and higher homologues.

Also the nature of the thiolgroup-bearing moiety seems to play some role on the in vitro antimicrobial activity. Thus, 2-[(trimethyltin)mercapto]benzoxazole (**5**) and 2-[(trimethyltin)mercapto]benzothiazole (**6**) were somewhat more active than trimethyltin lupinylsulfide (**1**) against some particular microorganisms.

These differences were not impressive and could render questionable the purported importance of the thiol group-bearing moieties, even though we supposed that the latter should appear in full in the in vivo investigations. However, much larger influences of the thiols used were observed in other cases, as in that of the dibutyltin compounds evaluated as trypanocides [9], indirectly supporting our working hypothesis.

Comparing triphenyltin lupinylsulfide (**4**) with the simple triphenyltin chloride, the latter appeared as ac-

tive as, or a little more active than the former against all tested strains, with the only exception of *C. albicans*. This is not surprising due to the fact that the tin atom in the latter compound is more exposed and prone to reaction; however, for the same reason, the triphenyltin chloride is less suitable than **4** for use as a therapeutic agent.

Concluding, some interesting antimicrobial activities were observed with the synthesized triorganotin compounds with a MIC as low as 0.06 μg/ml for one strain of *S. aureus* (**4**) and 1.95 μg/ml for *E. coli* (**2**). Considerable differences in susceptibility among the microorganisms were also observed for a given compound. Therefore, the prosecution of this study is justified with the investigation of the activity on some more microorganisms and the preparation of new tri- and diorganotin compounds derived from other thiols.

The study of all these compounds as anticancer agents will also be pursued.

Acknowledgements

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References

[1] F. Novelli, F. Sparatore, Thiolupinine and some derivatives of pharmacological interest, Farmaco 48 (1993) 1021–1049.

[2] F. Novelli, M. Recine, F. Sparatore, C. Juliano, Gold(I) complexes as antimicrobial agents, Farmaco 54 (1999) 232–236.

[3] A.K. Sijpesteijn, F. Rijkens, G.J.M. Van der Kerk, A. Manten, Antimicrobial activity of organogermanium derivatives, Nature 201 (1964) 736.

[4] M.H. Gitlitz, M.K. Moran, Tin compounds, in: Kirk-Othmer Encyclopedia of Chemical Technology, vol. 23, 3rd ed., Wiley, New York, 1983, pp. 42–77.

[5] A.K. Saxena, J.K. Koacher, J.P. Taudon, S.R. Das, Studies of organotin–Schiff base complexes as new potential amebicidal agents, J. Toxicol. Environ. Health 10 (1982) 709–715.

[6] N.F. Cardarelli, Controlled Release Pesticide Formulations, CRC Press, Boca Raton, FL, 1976.

[7] S.J. Blunden, P.A. Cusak, R. Hill, The Industrial Uses of Tin Chemicals, Royal Society of Chemistry, London, 1985.

[8] W. Peters, E.R. Trotter, B.L. Robinson, The experimental chemotherapy of leishmaniasis. VII. Drug responses of *L. major* and *L. mexicana* amazonensis, with an analysis of promising chemical leads to new antileishmanial agents, Ann. Trop. Med. Parasitol. 74 (1980) 321–335.

[9] J. Susperregui, A. Pestom, M. Bayle, G. Lain, C. Giroud, T. Baltz, G. Deleris, The in vitro trypanocidal activity of organotin compounds, Eur. J. Med. Chem. 32 (1997) 123–128.

[10] M. Gielen (Ed.), Tin-based Antitumor Drugs, Springer, Berlin, 1990.

[11] A.J. Crowe, The chemotherapeutic properties of tin compounds, Drug Future 12 (1987) 255–275.

[12] T.A.K. Al-Allaf, L.J. Rashan, W.F. Halasch, R.F. Khuzaie, Dimethyltin carboxylate compounds; synthesis, characterization and in vitro cytotoxic activity, Boll. Chim. Farm. 137 (1998) 55–58.

[13] M. Cagnoli, A. Alama, F. Barbieri, F. Novelli, C. Bruzzo, F. Sparatore, Synthesis and biological activity of gold and tin compounds in ovarian cancer cells, Anticancer Drugs 9 (1998) 603–610.