Long-Chain Derivatives with a Hexahydrothioxotetrazine Moiety as Potential Antimicrobial Agents

M.W.Y. Khan[a], J. Mustafa[a], A. Rauf[a], M.T. Saeed[b] and S.M. Osman[b,*]

[a]Applied Science Section, University Polytechnic, Faculty of Engineering and Technology, A.M.U., Aligarh 202 002, India and [b]Section of Oils and Fats, Department of Chemistry

A series of hexahydrothioxotetrazine-containing compounds I–IV were evaluated for their antimicrobial activity. Fungi (*Aspergillus sydowii, A. nidulari, A. terreus, Fusarium solanum, Trichoderma viridae, T. lignorum, Rhizopus arrhitus* and *Candida albicans*) and bacteria (*Escherichia coli, Salmonella* sp., *Pseudomonas aeruginosa, bacillus* sp., *Staphylococcus aureus, Klebsella* sp., *Shigella* sp. and *Streptococcus faecalis*) were used for this examination. Compounds 1–III showed 90% growth inhibition against a large number of fungi. Two compounds (II and III) have shown significant antibacterial activity. The most striking feature of this study is that compound II, which does not carry an ester function, is the most potent antifungal and antibacterial agent. In general, these promising compounds may be of commercial significance in the future and therefore need developmental studies.

KEY WORDS: Bacteria, fungi, hexahydrothioxotetrazine, microbes, microbial activity.

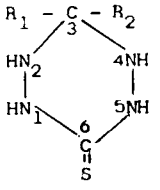
We previously have reported (1) a simple, efficient and quantitative method of preparation of the formerly unknown hexahydrothioxotetrazine nucleus incorporating fatty ester chains at different positions. This paper describes the antimicrobial (fungi and bacteria) studies of these valuable products. These may be developed as possible agrichemicals. This laboratory has already investigated antimicrobial activity of some nitrogen derivatives based on isoricinoleic acid (2), fatty morpholine compounds (3) and fatty tetrazoles (4).

MATERIALS AND METHODS

The fungi and bacteria used for microbial screening were obtained from the Indian Agricultural Research Institute (New Delhi, India) and from Jawaharlal Nehru Medical College, Aligarh Muslim University (Aligarh, India). These cultures were subcultured and maintained on potato dextrose agar (Hi media) and tryptone broth (Hi media) at 4°C. The following microorganisms were used: *Asper-*

TABLE 1

Antimicrobial Activity Against Fungi and Bacteria



	R_1	R_2
I	CH_3	$(CH_2)_8$-$COOCH_3$
II	CH_3	$(CH_2(_8$-CH_3
III	CH_3-$(CH_2)_5$	$(CH_2)_{10}$-$COOCH_3$
IV	CH_3-$(CH_2)_8$	$(CH_2)_7$-$COOCH_3$

	Growth inhibition at 1000 ppm (%)			
Organisms	I	II	III	IV
Fungi				
Aspergillus sydowii	90	90	90	60
A. nidulari	10	90	90	10
A. terres	05	90	50	40
Fusarium solanum	90	90	90	40
Trichoderma viridae	90	90	90	Nil[a]
T. Liqnorum	90	90	90	40
Rhizopus arrhitus	10	90	90	Nil
Candida albicans	90	90	90	Nil
Bacteria				
Escherichia coli	Nil	60	70	Nil
Salmonella sp.	10	60	70	Nil
Pseudomonas aeruginosa	Nil	60	60	Nil
Bacillus sp.	10	70	60	Nil
Staphylococcus aureus	20	70	10	Nil
Klebsella sp.	Nil	70	Nil	Nil
Shiqella sp.	Nil	80	70	Nil
Streptococcus faecalis	Nil	60	60	Nil

[a]Nil, zero inhibition.

*To whom correspondence should be addressed.

gillus sydowii, A. nidulari, A. terreus, Fusarium solanum, Trichoderma viridae, T. lignorum, Rhizopus arrhitus, Candida albicans, Escherichia coli, Salmonella sp., *Pseudomonas aeruginosa, Klebsella* sp. and *Streptococcus fecalis.*

EXPERIMENTAL PROCEDURE

Fatty compounds I–IV were prepared by a method detailed previously (1). Stock solutions (1%) of the test compounds I–IV were prepared in acetone for *in vitro* studies. Ten mL (1%) of each test compound was incorporated separately in 100 mL of autoclaved agar medium, so as to prepare 10 plates for each compound at 1000-ppm concentration. These treated plates were used as test plates. Acetone-incorporated plates served as control and tetracycline as a reference antibiotic. Prior to testing, the microorganisms (bacteria and fungi) were subcultured in liquid tryptone broth and in liquid potato dextrose for 24 h at 37°C and for 48 h at 28°C, respectively. These subcultures were seeded (3) on fresh, untreated plates and incubated for 24–48 h. Two-mm diameter discs were cut from these seeded plates and placed on treated agar plates. These treated plates with test organisms were incubated for 72 h at 28°C for fungi and at 37°C for bacteria. After 72 h, observations were taken and the radial growth was measured in all the plates and compared with control and reference antibiotic. The percent inhibition was calculated by the formula $DC - DT/TD \times 100 = \%$ inhibition, where DC was the radial growth in mm in control plates, DT was the radial growth in mm in treated plates, and TD was the total diameter of plates.

RESULTS AND DISCUSSION

Results of antimicrobial screening against two microbes are assembled in Table 1.

A great number of nitrogen- and sulfur-containing compounds have been investigated extensively because of their profound biological activities (2–8). The hexahydrothioxotetrazine group has both heteroatoms. Here, we report the antimicrobial activity of novel compounds, methyl hexahydro-3-methyl-6-thioxo-1,2,4,5-tetrazine-3-nonanoate (I), hexahydro-3-methyl-6-thioxo-1,2,4,5-tetrazine-3-nonane (II), methyl hexahydro-3-hexyl-6-thioxo-1,2,4,5-tetrazine-3-undecanoate (III) and methyl hexahydro-3-nonyl-6-thioxo-1,2,4,5-tetrazine-3-octanoate (IV). Compound I has shown remarkably high (90%) growth inhibition against all the fungi except for *A. nidulari* and *A. terreus*, and it is almost inert against bacteria. Compound II is most active against both the organisms, exhibiting 90% potentiality for fungi and 60–80% for bacteria. Compound III is as effective as compound II. Compound IV is absolutely impotent, even though it is very similar to compound III. These results, as well as the literature survey (2–8), help to show that activity of the compounds owe more to the substituent than to inherent activity of the ring, whether it is pyrazole, tetrazole, morpholinone or tetrazine.

ACKNOWLEDGMENTS

This program is financed in part by a grant from the USDA (PL-480). Authors J.M. and A.R. are grateful to Professor Ziaul Hasan, Principal, University Polytechnic, for research encouragement.

REFERENCES

1. Saeed, M.T., J. Mustafa, A. Rauf and S.M. Osman, *J. Am. Oil Chem. Soc. 69*:396 (1992).
2. Ahmed, S.M., F. Ahmad and S.M. Osman, *Ibid. 62*:1578 (1985).
3. Agarwal, R., M.H. Ansari, M.W.Y. Khan, M. Ahmad and K.D. Sharma, *Ibid. 66*:825 (1989).
4. Agarwal, R., A. Rauf, M.W.Y. Khan, M. Ahmad, S.M. Osman and K.D. Sharma, *J. Oil Tech. Assn. India 23*:22 (1991).
5. Upadhyay, J., U. Dave and H. Parekh, *J. Ind. Chem. Soc. 68*:413 (1991).
6. Mahajan, N.R., F.H. Havaldar and P.S. Fernandes, *Ibid. 68*:245 (1991).
7. Jolly, V.S., M.Y. Dalvi and A.K. Srivastava, *Ibid. 68*:513 (1991).
8. Dwivedi, B., N. Tiwari and Nizamuddin, *Ibid. 68*:515 (1991).

[Received March 5, 1992; accepted August 9, 1992]