RESEARCH PAPER

Accepted 2 July 1999
Received 27 August 1998
Indian J. Pharm. Sci. 1999, 61 (5) 271-274

Antimicrobial Activities of some 4H-1,2,4-triazoles

A.A. IKIZLER*, F. UCAR[1], N.D. DEMIRBAS, I. YASA[1], A. DEMIRBAS AND T. GENZER[1]
Department of Chemistry, Karadeniz Technical University, 61080 Trabzon, TURKEY
[1]Department of Biology, Faculty of Science, Ege University, 35100 Izmir, TURKEY

A series of 3,5-dialkyl-4-ethoxycarbonylamino-4H-1,2,4-triazoles and 3,5-dialkyl-4-tert-butoxycarbonylamino-4H-1,2,4-triazoles were tested for their *in vitro* antibacterial and antifungal activities in solid agar cultures against eight microorganisms. In tests, some of the compounds were shown to be very potent *in vitro* antifungal activity against the fungi used.

In recent years, a great deal of research has been devoted to the synthesis of certain 1,2,4-triazole derivatives and to the investigation of their various pharmacological properties including antifungal activities[1-11]. In this connection, the synthesis and antifungal activities of some, 1,2,4-triazoles have recently been reported by us[12-16]. In continuation of our interest in the synthesis and biological investigation of 1,2,4-triazole derivatives, we report here the testing results and fungicidal activities of some 4H-1,2,4-triazoles. The study deals with the *in vitro* screening for antifungal and antibacterial activities of a series of recently synthesized 3,4,5-trisubstituted-4-H-triazoles [I-IX]

EXPERIMENTAL

Synthesis:

Analogues of 3,5-dialkyl-14-ethoxycarbonylamino-4H,1,2,4-triazoles [I-IV] and 3,5-dialkyl-4-tert-butoxycarbonylamino-4H-1,2,4-triazoles [V-VII] were synthesized from the reactions of ester ethoxycarbonylhydrazones [X] and ester tert-butoxycarbonyl hydrazones [XI] respectively with monoacylhydrazines according to the recently described procedures[17,18]. Di(3-benzyl-4-ethoxycarbonylamino-4H-1,2,4-triazol-5-yl) methane [VIII] and di-(3-benzyl-4-tert-butoxycarbonylamino-4H-1,2,4-triazol-5-yl) methane [VIII] and di-(3-benzyl-4-tert-butoxycarbonylamino-4H-1,2,4-triazol-5-yl) methane (IX) were obtained from the treatment of malonic acid hydrazide with Xd and XId, respectively[17,18]. The starting compounds X and XI were prepared by the methods previously reported[19,20]. The required chemicals were obtained from Fluka. The purity of compounds screened in this work were checked with ^{1}H NMR (Varian 60A spectrometer) IR, (Perkin-Elmer 377 spectrophotometer) and a Buchi oil-heated melting point apparatus.

* For correspondence

Antimicrobial Screening:

The compounds were tested for *in vitro* antimicrobial activities against bacteria that included *Staphylococcus aureus* ATCC 6538P, *Escherichia coli* ATCC 11230, *Mycobacterium smegmatis* CCM 2067 and fungi that included *Trichophyton rubrum, Microsporum canis, Aspergillus flavus, Penicillum waksmani* and *Candida albicans* ATCC 18804[21,22]. These test microorganisms were obtained from the Basic and Industrial Microbiology Section, Department of Biology, Faculty of Science, Ege University and from Microbiology Section, Faculty of Medicine, Ege University, (Izmir, Turkey).

The antimicrobial activities of the compounds were determined with agar disk diffusion method[22,23] and mycelial growth inhibition tests were carried out by the agar diffusion method[21,24,25]. Due to insolubility in water, all the test compounds was dissolved in 95% ethanol.

In order to test the antimicrobial activity against the bacteria and yeast, each of the test bacteria and C. Albicans were inoculated into twenty five milliliters of Mueller

Table 1 - Antifungal activity of the test compounds

Compound no	Microorganisms (Colony diameters, mm)[a] *Trichophyton rubrum*	*Microsporum canis*	*Aspergillus flavus*	*Penicillium waksmanii*
Ia	23	20	20	9
Id	8	18	24	12
Ie	17	15	20	6
If	20	10	25	8
IIb	8	16	21	10
IIc	13	20	23	13
IId	12	14	19	11
IIf	10	13	24	11
IIIf	14	10	20	7
IVb	15	18	20	8
IVg	19	20	21	8
Va	12	11	20	6
Vd	10	14	25	5
VId	17	15	20	11
VIe	6	10	20	17
VIId	10	15	20	5
VIIe	20	11	20	8
VIII	11	11	20	7
IX	17	10	20	7
Ketoconazole	16	13	0	0
With ethanol	33	19	25	10
Untreated control	35	20	25	12

Colony diameters the test compounds I-IX, ketoconazole, ethanol and untreated control against dermatophytes mould fungi. [a]O=no growth Data are the average of n:5 experiments

Hinton Agar with 1 ml of 10^7 cells per ml respectively and filter paper disks containing the test compounds in a concentration of 70 µg/ml each were placed onto agar medium immediately after inoculation. Ethanol was used as control[26-31]. The test bacteria and *C. albicans* were incubated at 37° for two days, and *M. smegmatis* was incubated at 30°. The results, after substructing the ethanol zone diameter from the inhibition zone diameters of the test compounds were evaluated.

In order to test the antifungal activity for filamentous fungi, stock solutions were prepared with test compounds. One milliliter of these solutions were added to 25 ml of Sabouroud Maltose Agar, such that final concentration in the medium was adjusted to 70 µg/ml. Later, fungi carrying disks (5 mm) were cut from an actively growing colony of the test fungi, placed on the center of each petri dish and incubated at 37° and 27° for dermatophytes and moulds, respectively for one week. C. albicans was incubated at 37° for two days. Moreover, the azole derivative ketoconazole was used as a positive control in all assays[23,25,27,31,32].

Table 2 - Antifungal activity of compounds I-IX against *Candida albicans* ATTC 18804 with agar disk diffusion method

Compound no	Candida albicans Inhibition zone diameters (mm)
Ia	13
Id	8
Ie	8
If	13
IIb	13
IIc	8
IId	13
IIf	13
IIIf	14
IVb	14
IVg	8
Va	14
Vd	14
VId	10
VIe	12
VIId	12
VIIe	12
VIII	10
IX	8
Ketoconazole	5
With ethanol	5

Test results were evaluated according to the diameter of the mycelial mat of each fungus as compared with untreated control and alcohol control. The role of ethanol as a antimicrobial agent in agar diffusion method might not have any significance because it is used in very little amount (1 ml). Although in these experiments we used ethanol as a control, all of the experiments were repeated five times and the results were expressed as average values. Radial linear growth was determined by measuring the colony diameters. Growth inhibition was calculated as: radial growth without test compound-radial growth on ethanol = The effect of ethanol to the test microorganism. Similarly radial growth on ethanol-radial growth on test compound=The antimicrobial activity of test compound.

RESULTS

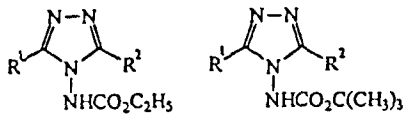
All the test compounds did not exhibit any activity against some standard strains of Gram-positive and Gram-negative bacteria. But, the antifungal activity of the test compounds was varied. Some of the tested compounds were shown to have very potent *in vitro* antifungal activity against dermatophytes (*T. rubrum* and *M. canis*) and yeast (*C. albicans*). The microbiological test results are given in Tables 1 and 2.

DISCUSSION

As seen from Table 1, ethanol did not affect the growth of test microorganisms. For this reason, the results were given as compared with untreated control. All of the test compounds showed very good activity and relatively good activity against *T. rubrum* as compared to the reference ketoconazole and untreated control, respectively. Also, compounds If, IIIf, Va, VIe, VIII and IX were found to be more effective than ketoconazole against *M. canis*. All the tested compounds showed no an important effect on *A. flavus*. But, compounds Ie, If, IIIf, IVb, IVg, Va, Vd, VIId, VIII and IX were relatively effective on *P. waksmanii*. Examination of Table 2 shows that the antifungal activities of compounds Ia, If, IIb, IId, IIf, IIIf, IVb, Va, Vd, VIe, VIId and VIIe are higher than that of the reference compound against *C. albicans*. In general, the most effective compounds are IIIf, IVb and Va. In spite of the fact that some recently reported 4-arylideamino-4,5-dihidro-1H-1,2,4-triazole-5-ones generally exhibited good activity against *A. flavus* and *P. waksmanii*, 1,2,4-triazole derivatives tested in study generally showed good activity against *T. rubrum, M. canis* and *C. albicans*.

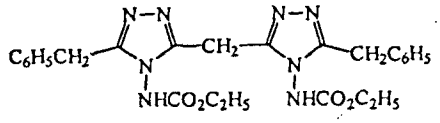
REFERENCES

1. Van Gestel J., Heeres, J., Jonssen, M. and Van Reet, G., **Pestic Sci.**, 1980, 11, 95.
2. Trzhsinskaya, B.V., Skvortsova, G.G., Mansurov Yu. A., Buchin, P.I. and Viderker, M.G., **Khim. Farm. Zh.**, 1982, 16, 1466.
3. Shephard, M.C., Noon, R.A., Worthington, P.A., Mccellan, W.D. and Lever., B.C., **Pest Dis.**, 1986, 1, 19.
4. Leroux, P., **Phytoma**, 1987, 31, 6.
5. Fujimoto, T.T, Quinn, J.A., Egan, A.R., Shaber, S.H. and Ross, R.R., **Pestic. Biochem. Physiol.**, 1988, 30, 199.
6. Chollet, J.F., Bonnemain, J.L., Miginiac, L. and Rohr, P, **Pestic Sci.**, 1990, 29, 427.
7. Hata, T., Frukawa, Y., Konosu T. and Qida S., **Bull. Chem. Soc. Jpn.**, 1991, 64, 2877.
8. Murabayashi, A., Masuko, M., Niikawa M., Shriane, N., Furuta, T., Hayashi, Y. and Makusimi, Y.J., **Pesticide Sci.**,

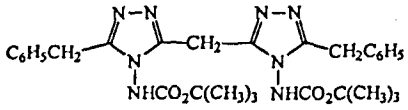


I-IV

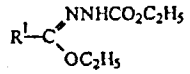
V-VII



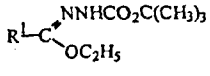
VIII



IX



X



XI

	R^2
I, V	CH_3
II, VI	3-pyridyl
III, VII	4-pyridyl
IV	2-furyl

	R^1
a	CH_3
b	CH_2CH_3
c	$CH_2CH_2CH_3$
d	$CH_2C_6H_5$
e	$CH_2C_6H_4.Cl(p\text{-})$
f	C_6H_5
g	$C_6H_4.CH_3(p\text{-})$

Structures

1991, 16, 419.

9. Bansal, O.P., Srinivas, J.S., and Sastry, C.V., **Indian J. Chem.(B),** 1992, 31, 289.
10. Krimer, M.Z., Tachchi, V.P., Kal'yan Yu, B., Makaev, F.Z., Putsykin, Yu, G. and Molchanov, O.Y., **Khim.-Farm. Zh.** 1995, 29, 24.
11. Miyauihi, H., Kozuki, K., Tanio, T. and Ohashi. N., **Chem. Pharm. Bull.** 1996, 44, 785.
12. Demirbas, A., Johanson, C.B., Duman, N. and IKizler, A.A., **Acta Polon. Pharm-Drug Research,** 1996, 53, 117.
13. Dogan, N., Ikizler A., Johanson, C.B. and Ikizler, A.A., **Acta Polon. Pharm-Drug Research,** 1996, 53, 227.
14. Yuksek, H., Demirbas, A., Ikizler, A., Johanson, C.B., Celik, C. and Ikizler, A.A., **Arzneim.-Forsh-Drug Res.,** 1997, 47, 405.
15. Ikizler, A.A., Ikizler, A., Serdar, M. and Yildirim, N., **Acta Polon, Pharm-Drug Research,** 1997, 54, 363.
16. Ikizler, A.A., Ucar, F., Yuksek, H., Aytin, A., Yasa I. and Genzer, T., **Acta Polon. Pharm.-Drug Research,** 1997, 54, 135.
17. Ikizler, A., Demirbas, A. and Ikizler, A.A., **Polish J. Chem.** 1996, 70, 1114.
18. Ikizler, A., Demirbas, N. and Ikizler, A.A., **J. Heterocyclic Chem.,** 1996, 33, 1765.
19. Un, R. and Ikizler, A., **Chim. Acta Turc.,** 1975, 3, 313; **Chem Abstr.,** 1976, 84, 150568g.
20. Ikizler, A.A., Ikizler, A., Yuksek, H., Bahceci, S. and Sancak, K., **Tr. J. Chem.** 1994, 18, 51.
21. Isaaccson, D.M. and Platt, T.B., In; O'Leary, W.M. Eds, Practical Handbook of Microbiology, CRC Press. Inc.,. Boca Raton, Florida, 1989, 621.
22. Pogwizd, S.M. and Lerner, S.A., In; O'Leary, W.M. Eds, Practical Handbook of Microbiology, CRC Press. Inc., Boca Raton, Florida, 1989, 603.
23. Torttoro, G.J., Funke, B.R. and Case, C.L., In; Microbiology, The Benjamin Cummings Publishing Company, Inc., 390 Bridge Parkway, 1995, 491.
24. Berdy, J., In; Bushell, M.E. and Grafe, V. Ed., Bioactive Metabolites from Microorganisms, Elsevier Science Publishers, Amsterdam, 1989, 3.
25. Bianchi, D., Cest, P., Spezia S., Gravalgia, C. and Mirenna, V., **J. Agric. Food Chem.,** 1991, 39, 197.
26. McKeen, C.D., Reily, C.C. and Pusey, P.I., **Phytopathology,** 1986, 76, 136.
27. Moller, N.F., Hrass, E., Hojbjerg, T., Moller, S., Mortensen, I. and Thomsen, V.F., **Microbial. Immunol. Scand. B.,** 1986, 94, 159.
28. Mullen G.B., Swift, P.A., Marinyak, D.M., Allen, S.D., Mitchell, J.T., Kinsolving, C.R. and Georgiev, V. St., **Helv. Chim. Acta,** 1988, 71, 118.
29. Krup, A., Kumar, V. and Rao, M.N.A., **Pharmazie,** 1989, 44, 870.
30. Marinyak, D.M., Mullen G.B., Allen, S.D., Mitcell, J.T., Kinsolving C.R. and Georgiev, V. St., **Arzneim.-Forsch. Drug Res.** 1990, 40, 95.
31. Murabayashi, A., Masuko, M., Niikawa, M., Stirane, N., Furuta, T., Hayashi, Y. and Maksimi, Y., **J. Pesticide Sci.,** 1991, 16, 419.
32. Zaika, L.L. **J. Food Safety,** 1988, 9, 97.