Activity of a Series of β-Lactams against Some Phytopathogenic Fungi

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A series of 4-(aryloxy)- and 4-(arylthio)azetidin-2-ones were synthesized by reacting 4-acetoxyazetidin-2-one with phenols or thiophenols and by subsequent alkylation at the nitrogen. The compounds were tested against phytopathogenic fungi of different taxonomic classes in vitro and in vivo. Some of these compounds showed antifungal activity, in particular against *Phycomycetes*. In general, ethers are slightly more active than thioethers.

INTRODUCTION

In a previous paper (Arnoldi et al., 1988) we reported that some 4-(aryloxy)- and 4-(arylthio)azetidin-2-ones had antimycotic activity in vitro against various species of *Microsporum*, *Trichophyton*, and *Candida*. In the best cases the minimum inhibitory concentrations were in the range 2–8 mg L^{-1}.

The results had some interest because although an enormous number of β-lactams that exhibit antimicrobial activity have been isolated or synthesized, only a few of them are antifungal (Brown et al., 1979; King et al., 1986; Wanning et al., 1981; Pruess and Kellet, 1983; Mueller et al., 1983).

As a Japanese patent (Ube Industries, 1981) claims fungicidal activity of a series of substituted azetidin-2-ones against the plant pathogen *Pyricularia oryzae*, we decided to test our compounds also against some phytopathogenic fungi of different taxonomic classes.

The compounds prepared and tested have the structures shown in Chart I and Table I.

The azetidin-2-ones **1–22** (structure A) were obtained in moderate to good yields by condensation of the appropriate phenol or thiophenol with the easily available 4-acetoxyazetidin-2-one (**26**) (Clauss et al., 1974) or 4-(benzoyloxy)azetidin-2-one in the presence of a base. Compound **1** was obtained by refluxing propargyl alcohol and 2-(benzoyloxy)azetidin-2-one in benzene in the presence of $Zn(OAc)_2$.

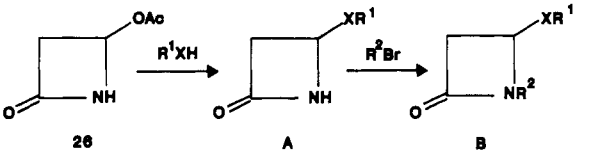
In some cases a CH_2CO_2R or $CH_2C{\equiv}CH$ group was introduced onto the β-lactam nitrogen of compound A to verify the importance of the free NH group for the activity and to increase the lipophilicity, thus giving compounds with structure B. The alkylation was performed with alkyl bromides on the sodium salts of the azetidinones in tetrahydrofuran (THF) and dimethylformamide (DMF) at −60 °C. Only some examples of the most important synthetic procedures will be reported under Experimental Procedures.

EXPERIMENTAL PROCEDURES

Synthesis of Compounds. Details of the analytical characterization of the compounds were already reported (Arnoldi et al., 1988). 4-(Benzoyloxy)azetidin-2-one was purchased from Aldrich.

Synthesis of Compound 1. Method A. In a flask equipped with a Dean-Stark and a $CaCl_2$ valve, $Zn(OAc)_2$ (0.85 g, 3.87 mmol) was refluxed in benzene (30 mL) for 1 h. Propargyl alcohol (1.32 g, 23.25 mmol) and 4-(benzoyloxy)azetidin-2-one (1.5 g, 7.75 mmol) were added, and the mixture was refluxed for 23 h. It was then cooled, filtered, and concentrated. The oily residue was purified by column chromatography (hexane/ethyl acetate 1:1) to give 900 mg of **1** as a colorless oil, 92% yield. Anal. Calcd for $C_6H_7NO_2$: C, 57.59; H, 5.64; N, 11.20. Found: C, 57.15; H, 5.58; N, 11.04. NMR δ 2.49 (1 H, t, J = 2 Hz, C≡CH), 2.8–3.4 (2 H, AB of ABX, H-3), 4.25 (2 H, d, J = 2 Hz, $OCH_2C{\equiv}C$), 5.21 (1 H, X of ABX, H-4), 6.5 (1 H, br, NH).

Chart I



26 A B

Synthesis of Compound 13. Method B. 4-Acetoxyazetidin-2-one (1 g, 7.75 mmol) and 2-mercaptobenzyl alcohol (Arnoldi and Carughi, 1988) were dissolved in 5 mL of dioxane. Sodium hydroxide (280 mg, 7.75 mmol) in water (2.5 mL) was added in 1 h at 0 °C. The mixture was then stirred for 2 h, diluted with water (5 mL), and extracted with ethyl acetate (4 × 5 mL). The organic layer was dried and concentrated. The brown oily residue was then crystallized from toluene, 0.74 g, 46% yield: mp 58–60 °C (toluene). Anal. Calcd for $C_{10}H_{11}NO_2S$: C, 57.39; H, 5.29; N, 6.69. Found: C, 57.77; H, 5.39; N, 6.65. NMR δ 2.46 (OH), 2.93 (1 H, A of ABX, J = 15 and 5 Hz, H-3), 3.35 (1 H, B of ABX, J = 15 and 5 Hz, H-3), 4.80 (2 H, s, CH_2O), 4.90 (1 H, X of ABX, J = 3 and 5 Hz, H-4), 6.73 (NH), 7.2–7.7 (4 H).

Synthesis of Compound 24. Method C. Compound **14** (0.4 g, 1.9 mmol) in DMF (1 mL) was added to a slurry of NaH (80% in paraffin oil, 135 mg, 4.5 mmol) in anhydrous DMF (6 mL) and THF (6 mL) at −60 °C. After 0.5 h at the same temperature, ethyl bromoacetate (0.32 mL) was added. The mixture was stirred 1 h at −50 °C and then poured in water (60 mL) and extracted with ethyl acetate (3 × 40 mL). After drying, the solvent was evaporated to give a crude oil which was purified by column chromatography, 340 mg, 60% yield. Anal. Calcd for $C_{14}H_{17}NO_4S$: C, 56.93; H, 5.80; N, 4.74. Found: C, 57.12; H, 5.75; N, 4.81. NMR δ 1.25 (3 H, t, J = 7 Hz, CH_3), 2.83 (1 H, A of ABX, H-3), 3.33 (1 H, B of ABX, H-3), 3.78 (1 H, d, J = 18 Hz, NCHCO), 3.80 (3 H, s, OCH_3), 4.15 (2 H, q, J = 7 Hz, CH_2), 4.25 (1 H, d, J = 18 Hz, NCHCO), 5.08 (1 H, X of ABX, J = 5 and 2 Hz, H-4), 6.85 (2 H, m, H-3′), 7.33 (2 H, m, H-2′).

Test Fungi. Pathogenic strains of the following fungi were used: *Botrytis cinerea* Pers. on malt agar, *Colletotrichum lindemuthianum* Sacc. et Magn. on neopeptone yeast extract glucose agar, *Phytophthora infestans* (Mont.) De Bary on V8 juice agar. *Sphaerotheca fuliginea* (Sch.) Salmon, *Plasmopara viticola* (Berk and Curtis ex De Bary) Bel. and de Toni, and *Uromyces appendiculatus* (Pers.) Link were maintained on stock plants.

Test for Fungitoxicity in Vitro. The compounds were assayed against mycelial growth by the commonly used poisoned

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Table I. Physicochemical Data and Biological Activity of Compounds Expressed as ED_{50} (Milligrams per Liter)

compd	XR^1	R^2	synthetic method	yield, %	mp, °C	in vitro tests[a] B	C	P	in vivo tests:[b] protectant activity[c] P/V	P/S	B/grapes	C/P
1	$OCH_2C{\equiv}CH$	H	A	92	oil	>100	>100	80	>500			
2	OC_6H_4-4-COPh	H	B	47	107	>100	100	17	>500			
3	OC_6H_4-4-Ph	H	B	25	153	>100	>100	>100				
4	O-2-naphthyl	H	B	54	114	50	25	23	350		250	>1000
5	O-1-naphthyl	H	B	31	127–129	60	100	>100				
6	OC_6H_4-4-$COCH{=}CHPh$	H	B	70	139–143	100	100	60	250			
7	OC_6H_4-4-$COCH_2CH_2Ph$	H	*d*	60	60–65	>100	100	100				
8	OC_6H_4-4-$COCH{=}CHC_6H_4$-4-OMe	H	B	45	200–205	70	>100	>100				
9	O-9-phenanthryl	H	B	20	145–147	>100	>100	>100				
10	SPh	H	B	32	58–60	17	100	35	>500	>500		
11	SC_6H_4-2-CO_2H	H	B	84	181	>100	>100	>100				
12	SC_6H_4-4-Cl	H	B	42	104	75	100	100				
13	SC_6H_4-2-CH_2OH	H	B	46	58–60	>100	>100	>100				
14	SC_6H_4-4-OMe	H	B	11	74	45	>100	80	500	>500	>500	
15	S-2-naphthyl	H	B	73	112–114	100	85	13	300	>500		>1000
16	S-2-pyridinyl	H	B	45	oil	>100	>100	10	>500	>500		
17	S-4-pyridinyl	H	B	35	116	>100	>100	35	>500	>500		
18	S-2-pyrimidinyl	H	B	40	133–135	>100	>100	>100				
19	S-2-(1-methylimidazolyl)	H	B	30	115	>100	>100	80	>500	>500		
20	S-2-benzothiazolyl	H	B	24	123–125	50	17	16	500	>500	>500	1000
21	S-5-(1-phenyltetrazolyl)	H	B	11	129–134	>100	>100	>100				
22	SO_2-2-naphthyl	H	B	57	192–196	>100	>100	>100				
23	SC_6H_4-4-Cl	$CH_2C{\equiv}CH$	C	17	oil	>100	75	>100				1000
24	SC_6H_4-4-OMe	CH_2CO_2Et	C	60	oil	>100	>100	>100				
25	S-2-naphthyl	CH_2CO_2Et	C	44	oil	>100	37	10	>500			>1000

[a] B, *B. cinerea*; C, *C. lindemuthianum*; P, *P. infestans*. [b] P/V, *P. viticola* on *V. vinifera*; P/S, *P. infestans* on *S. lycopersicum*; B/grapes, *B. cinerea* on grapes; C/P, *C. lindemuthianum* on *P. vulgaris*. [c] All the compounds were tested in vivo against *U. appendiculatus* on *P. vulgaris* and *S. fuliginea* on *C. sativus*. They were inactive, except 12 which had ED_{50} = 850 on the former. [d] Obtained by catalytic reduction of compound 6.

food technique (Arnoldi et al., 1989) at the concentration of 100, 50, 25, and 12.5 mg L^{-1}.

Activity was expressed as the percentage of inhibition of growth compared with the control (no chemical). These values were plotted on a probit scale against chemical concentrations to obtain 50% inhibition concentrations (ED_{50}).

Tests for Fungitoxicity in Vivo. Tests were performed on the following pathogen–host combinations: *S. fuliginea*/*Cucumis sativus*, *U. appendiculatus*/*Phaseolus vulgaris* L., and in some cases *C. lindemuthianum*/*P. vulgaris* L., *P. viticola*/*Vitis vinifera* L., and *P. infestans*/*Solanum lycopersicum* L.

Test plants were grown in pots containing sterilized soil and maintained either in a greenhouse or a growth room (temperature 22–23 °C, relative humidity 75–100%).

Direct protectant activity was assayed by spraying suspensions of the compounds at the concentrations of 1000, 500, 250, and 125 mg L^{-1} on both surfaces of the plant leaves. Inoculation was performed 24 h after treatment (Arnoldi et al., 1989).

Postinfectional activity was carried out on *P. viticola*/*V. vinifera* by applying the compounds 24 h after inoculation.

The compound activity was calculated on the basis of the percentage inhibition of the disease in comparison with the inoculated untreated plants. The ED_{50} values were estimated from dosage–response curves as indicated above.

RESULTS AND DISCUSSION

The results of the activity tests are reported in Table I.

Considering active the compounds with $ED_{50} \leq 50$, four of them (**4**, **10**, **14**, **20**) were inhibitory in vitro of *B. cinerea*, three (**4**, **20**, **25**) of *C. lindemuthianum*, and eight (**2**, **4**, **10**, **15–17**, **20**, **25**) of *P. infestans*. *Phycomycetes*, therefore, appear to be the most sensitive fungi. Only two compounds (**4** and **20**) inhibit the growth of all the three fungi, while two (**10** and **25**) are active on two.

All of the compounds were tested against *U. appendiculatus* on *P. vulgaris* and *S. fuliginea* on *C. sativus*, but none of them were active in these two tests. The compounds that were active against one or more pathogens in vitro were tested also on the same or on taxonomically similar pathogens in vivo. Some of them showed a protectant activity against downy mildew of grapes. The best compound was **6** (which gave less activity in vitro), followed by compounds **15** and **4**. None of them, however, reduced the infection when applied after inoculation. Compound **4** exhibited activity also against *B. cinerea* on grapes. The others were inactive.

From the point of view of structure, these compounds can be divided in two classes: the ethers and the thioethers. Among the ethers (**1–9**) compound **4** containing the 2-naphthyl group was the most active. The position of the substitution is very important, because compound **5**, which contains the 1-naphthyl group, is much less active. The activity of compound **6** was lost with the modification introduced to obtain compounds **7** and **8**.

In the thioethers (**10–21**) compound **15** with the 2-naphthyl substituent and compound **20** with the 2-benzothiazolyl group are the most active. Compounds **16** and **17** containing the pyridine ring were active, while compound **18** containing the pyrimidine nucleus was completely inactive. The only sulfone prepared (**22**) was devoid of activity.

Compounds **23–25** are thioethers in which the β-lactam nitrogen has been alkylated. Only compound **25** was more active than the parent compound.

The data of Table I were compared with the results obtained on different species of *Trichophyton*, *Microsporum*, and *Candida* (Arnoldi et al., 1988). With the exception of compound **12** the compounds with the wider spectrum of activity on human mycoses (such as **4**, **6**, **10**, **15**, **16**, and **25**), in general, were the most active also on the phytopathogenic fungi. In the case of *T. mentagrophytes* a QSAR study showed that steric parameters were more important than lipophilicity in determining the activity of the series. Similar studies were not performed with the plant pathogens.

In conclusion, some synthesized compounds have a moderate activity against phytopathogenic fungi. Nothing is known about the mechanism of action of this class, while

the action of fungitoxic clavams seems to be due to an inhibition of methionine biosynthesis (Pruess and Kellet, 1983).

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