Synthesis and Antifungal Activity of Some New Benzimidazole Derivatives

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Synthesis and antifungal evaluation of 5-ethoxycarbonyl-2-(substituted-benzyl or phenoxymethyl)benzimidazoles are reported. Structures of the compounds were elucidated with IR-, ^{1}H-NMR-, ^{13}C-NMR-, mass-spectra and elemental analysis. Preliminary results show that none of the synthesized benzimidazole derivatives has antifungal activity at the concentration of 100 µg/ml against *Candida parapsilosis, Candida stellatoidea, and Candida pseudotropicalis.*

Synthese und antimykotische Wirkung einiger neuer Benzimidazol-Derivate

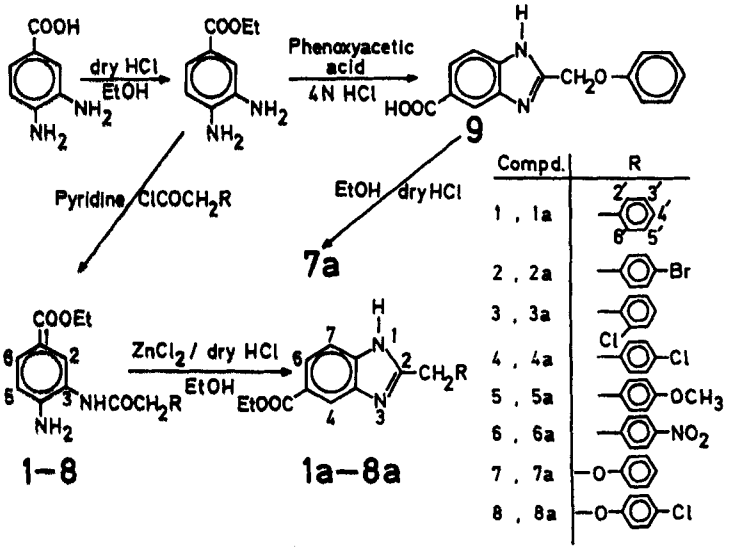
Synthese und antimykotische Prüfung einiger 5-Ethoxycarbonyl-2-(subst. benzyl oder phenoxymethyl)benzimidazole werden beschrieben. Die Strukturen wurden durch IR-, ^{1}H-NMR, ^{13}C-NMR und Massenspektren sowie Elementaranalysen gesichert. Erste Resultate zeigen, daß diese Benzimidazolderivate keine Aktivität gegen *Candida albicans, C. parapsilosis, C. Stellatoidea und C. pseudotropicalis* in der Konzentration 100 µg/ml haben.

Since the benzimidazole ring was shown to inhibit the growth of certain yeasts[1] many benzimidazole compounds have been synthesized[2,3]. However, as resistance to benzimidazoles is developed within a short period of time, the use of benzimidazoles as agricultural fungicides was given up recently[4]. The imidazoles, 1,2,4-triazoles, and pyrimidines which were prepared by chemical variations of benzimidazoles are most important antimycotica and agricultural fungicides, respectively[3].

In the present paper, the synthesis and antifungal activity of new simple benzimidazole derivatives are described.

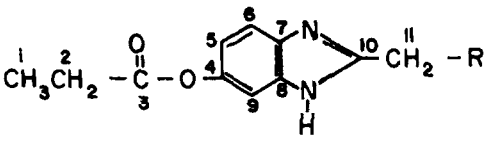
Results and Discussion

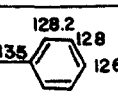
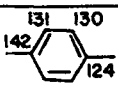
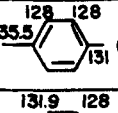
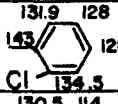
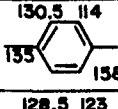
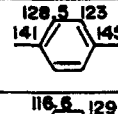
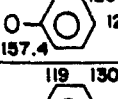
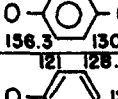
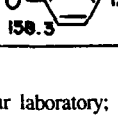
The synthesis of the benzimidazole compounds involved two steps: 1) preparation of mono amide derivatives by reaction of ethyl 3,4-diaminobenzoate with chlorides of substituted phenyl or phenoxyacetic acids; 2) preparation of the final products, by dehydration of the intermediates **1-8**, with anhydrous $ZnCl_2$ and dry HCl gas. Only compound **9** was obtained by *Phillips'* method[5] in very low yield. - ^{13}C-NMR shift assignments of compounds **1a-8a** and **9** are given in Tab. 1.



  0365-6233/91/0505-0283 $ 3.50 + .25/0

Table 1: ^{13}C-chemical shift assignment of compounds **1a-9**



C number / Comp. number	1	2	3	4	5	6	7	8	9	10	11	R
1 a	14.2	32.3	165.2	133.7	126	114.3	135	133	115.4	155	60.3	
2 a	13.9	32.3	165.3	135	127	114.4	135	132	115.7	154	61.35	
3 a	14.5	31.4	165.5	136.6	127.2	114.7	135.4	132.5	115.9	154.8	61.3	
4 a	13.5	31.7	165	133.7	127	114.3	136.8	132.8	115.4	154.2	61.5	
5 a	14.2	31.6	165	135	126.09	114.5	135	132	115.4	155.7	61.3	
6 a	14.3	33	162	135.6	126.9	115.1	135.6	132.9	116.9	152.2	61.8	
7 a	14.3	33	165	132.8	126.6	114.7	135.7	132.7	115.2	154.7	62.2	
8 a	14.6	28.8	169	133.8	126.0	115.6	135.7	130.1	114.1	155.9	62.7	
9	-	-	166.4	130	125.1	115.2	130.1	130	115.3	153.3	64	

Antifungal activities of compounds **1a-8a**, and **9** against *Candida parapsilosis, C. stellatoidea, C. albicans, and C. pseudotropicalis* were studied by liquid dilution in the test-tube (micrograms per ml)[6]. Since the compounds were insufficiently soluble in water, all compounds were dissolved in DMSO (1 mg/ml) and then final concentrations were adjusted to 100 µg/ml, 50 µg/ml, 25 µg/ml by using liquid medium and in the presence of the pertinent fungus suspension. Final incolum of microorganism was realized as 10^{-5}-10^{-6} cells/ml. Higher doses were not used because of the influence of DMSO. None of the compounds was active even at 100 µg/ml doses against the fungi mentioned above.

Experimental Part

Melting points: Büchi SMP-20 capillary m.p. apparatus, uncorrected. - CHN-microanalyses: Hewlett-Packard 185 C,H,N analyser. - ^{1}H-NMR (80,13 MHz) and ^{13}C-NMR (20,1 MHz) spectra: Bruker AC-80 spectrometer, d_6-DMSO, TMS as internal standard, δ (ppm) scale. - Mass spectra: Varian MAT CH 7. - IR (KBr) SP-1100 Pye-Unicam. All N-C=O-bands at *1655-1665* cm^{-1}, all O-C=O-bands at *1730-1735* cm^{-1}, *if not stated* otherwise.

Ethyl 3,4-diaminobenzoat[7] was prepared in our laboratory; 3,4-diaminobenzoic acid from Merck, Fluka; phenylacetic acid derivatives from Aldrich. *Sabouraud* Dextrose Broth (Oxoid) liquid media was used for the antifungal activity.

Method A

The related phenoxyacetic acids or phenylacetic acids (7.35 mmol) were refluxed in benzene (5 ml) with $SOCl_2$ (2.5 ml) for 60 min at 80°C. Then solvent and excess of $SOCl_2$ were evaporated and ethyl 3,4-diaminobenzoate (7.5 mmol in 15 ml of benzene) and 7.35 mmol of pyridine were added. The mixture was refluxed for 3 h. Removal of the solvent gave a residue which was crystallized from $CHCl_3$/EtOH (2:8) to give compounds **1-8**. Melting points and yields of these intermediates are given below.

^{1}H-NMR spectra of **1-8**: 1.30-1.40 (t, 3H, J = 7 Hz, CH_2CH_3), 4.25-4.30 (q, 2H, J = 7 Hz, CH_2CH_3), 3.65-4.75 (s, 2H, $COCH_2$ and OCH_3 protons of compound **5**), 6.80-8.1 (aromatic protons), 8.1-8.2 (s, 1H, NHCO), 9.70 (s, 2H, NH_2). The integral values support the proton number.

Method B

To a mixture of 2 mmol of comp. **1-8** and absol. EtOH (20 ml) were added 11 mmol freshly prepared anhydrous $ZnCl_2$; after dissolving all $ZnCl_2$, dry HCl gase (approximately 1.5 g) was passed through the clear

Comp. No	1	2	3	4	5	6	7	8
m.p. °C	170	201	203	195	165	248	168	213
Yield (%)	27.4	51.2	31.8	47.2	45.6	31.5	48.3	57.3

solution which was refluxed until the starting materials were used up (at least 3 h). Then, ethanol was evaporated, dilute NH_3 solution was added, and the mixture was extracted with $CHCl_3$. The chloroform solution was washed with water 3 times, dried (Na_2SO_4) and evaporated.

The oily residue was dissolved in EtOH, 0.02 ml HCl acid (25 %) were added and the mixture was stirred vigorously. After addition of ether, the HCl salts of the benzimidazole derivatives **1a-8a** precipitated.

2-Phenylmethyl-5-[1H]benzimidazole carboxylic acid ethyl ester HCl (**1a**)

1a was obtained from compound **1** as white crystals, m.p. 233°C, yield 47.3 %. - ^{1}H-NMR: 1.40 (t, J = 7 Hz, 3H, CH_2CH_3), 4.40 (q, J = 7 Hz, 2H, CH_2CH_3), 4.60 (s, 2H, CH_2), 7.25-7.6 (m, 5H, phenyl), 7.9 (dd, $J_{7,6}$ = 8 Hz, $J_{7,4}$ = 0.7 Hz, 1H, H-7), 8.1 (dd, $J_{6,7}$ = 8 Hz, $J_{4,6}$ = 1.4 Hz, 2H, H-4,6), 8.30 (s, 1H, N^1-H), 8.75 (broad s, N^+-H). - MS (70 eV) m/z: 280 (100 %, M^+), 251 (13), 235 (50), 207 (13), 91 (8). - $C_{17}H_{17}O_2N_2Cl$ (316.5) Calcd. C 64.5 H 5.37 N 8.85 Found C 64.8 H 5.10 N 9.10.

2-(4-Bromophenyl)methyl-5-[1H]benzimidazole carboxylic acid ethyl ester HCl (**2a**)

2a was obtained from **2** as white needles, m.p 255°C, yield 43 %. - ^{1}H-NMR: 1.40 (t, J = 7 Hz, 3H, CH_2CH_3), 4.40 (q, J = 7 Hz, 2H, CH_2CH_3), 4.60 (s, 2H, CH_2), 7.55 (s, 4H, H-2',3',5',6'), 7.8 (dd, $J_{7,6}$ = 8 Hz, $J_{7,4}$ = 0.7 Hz, 1H, H-7), 8.1 (dd, $J_{6,7}$ = 8 Hz, $J_{4,6}$ = 1.4 Hz, 2H, H-4,6), 8.25 (s, 1H, N^1-H), 9.70 (braod s, N^+-H). - MS (70 eV) m/z: 360 (100 %, M^+), 358 (100, M^+), 345 (12), 343 (12), 331 (16), 329 (16), 315 (56), 313 (56), 250 (12), 207 (28), 172 (36), 170 (36), 118 (44). - $C_{17}H_{16}O_2N_2BrCl$ (395.5) Calcd. C 51.6 H 4.05 N 7.08 Found C 51.5 H 4.05 N 7.50.

2-(2-Chlorophenyl)methyl-5-[1H]benzimidazole carboxylic acid ethyl ester HCl (**3a**)

3a was obtained from **3** as white needles, m.p 216°C, yield 38 %. - ^{1}H-NMR: 1.40 (t, J = 7 Hz, 3H, CH_2CH_3), 4.40 (q, J = 7 Hz, 2H, CH_2CH_3), 4.6 (s, 2H, CH_2), 7.25-7.7 (m, 4H, H-3',4',5',6'), 7.8 (dd, $J_{7,6}$ = 9 Hz, $J_{7,4}$ = 0.7 Hz, 1H, H-7), 8.1 (dd, $J_{6,7}$ = 9 Hz, $J_{4,6}$ = 1.45 Hz, 2H, H-4,6), 8.30 (s, 1H, N^1-H), 6.75 (s, N^+-H). - MS (70 eV) m/z: 316 (4 %, M^+), 314 (12, M^+), 280 (100), 272 (5), 270 (16), 265 (12), 251 (32), 206 (30), 127 (5), 125 (15), 118 (17), 91 (8). $C_{17}H_{16}O_2N_2Cl_2$ (351) Calcd. C 58.1 H 4.56 N 7.98 Found C 58.0 H 4.63 N 8.25.

2-(4-Chlorophenyl)methyl-5-[1H]benzimidazole carboxylic acid ethyl ester HCl (**4a**):

4a was obtained from **4** as white crystals, m.p 252°C, yield 44.4 %. - ^{1}H-NMR: 1.40 (t, J = 7 Hz, 3H, CH_2CH_3), 4.40 (q, J = 7 Hz, 2H, CH_2CH_3), 4.6 (s, 2H, CH_2), 7.45 and 7.6 (2d, $J_{2',3'}$ and $J_{5',6'}$ = 12 Hz, A_2X_2, 4H, H-2',3',5',6'), 7.8 (d, $J_{7,6}$ = 8 Hz, 1H, H-7), 8.1 (dd, $J_{6,7}$ = 8 Hz, $J_{4,6}$ = 1.45 Hz, 2H, H-4,6), 8.30 (s, 1H, H-7), 10.4 (braod s, N^+-H). - MS (70 eV m/z): 316 (33 %, M^+), 314 (100, M^+), 301 (3), 299 (10), 287 (5), 285 (15), 271 (17), 269 (52), 250 (9), 243 (4), 241 (11.5), 128 (10), 126 (30), 118 (10), 91 (9). - $C_{17}H_{16}O_2N_2Cl_2$ (351) Calcd. C 58.1 H 4.56 N 7.98 Found C 57.9 H 4.63 N 8.20.

2-(4-Methoxyphenyl)methyl-5-[1H]benzimidazole carboxylic acid ethyl ester HCl (5a)

5a was obtained from **5** as white needles, m.p 218°C, yield 47.6 %. - ^{1}H-NMR: 1.40 (t, J = 7 Hz, 3H, CH_2CH_3), 3.7 (s, 3H, OCH_3), 4.40 (q, J = 7 Hz, 2H, CH_2CH_3), 4.50 (s, 2H, CH_2), 6.9 and 7.45 (2d, $J_{2',3'}$ and $J_{5',6'}$ = 9 Hz, A_2X_2, 4H, H-2',3',5',6'), 7.8 (d, $J_{7,6}$ = 8 Hz, 1H, H-7), 8.1 (dd, $J_{6,7}$ = 8 Hz, $J_{4,6}$ = 1.45 Hz, 2H, H-4,6), 8.30 (s, 1H, N^1-H). - MS (70 eV) m/z: 310 (100 %, M^+), 295 (29), 265 (29), 221 (8), 118 (10). - $C_{18}H_{19}O_3N_2Cl$ (346.5) Calcd. C 62.34 H 5.48 N 8.08 Found C 62.2 H 5.90 N 8.15

2-(4-Nitrophenyl)methyl-5-[1H]benzimidazole carboxylic acid ethyl ester HCl (**6a**)

6a was obtained from **6** as white crystals, m.p 269°C, yield 36 %. - ^{1}H-NMR: 1.40 (t, J = 7 Hz, 3H, CH_2CH_3), 4.40 (q, J = 7 Hz, 2H, CH_2CH_3), 4.70 (s, 2H, CH_2), 7.25 (broad s, N^+-H), 7.7 and 7.9 (2d, $J_{2',3'}$ and $J_{5',6'}$ = 3.5 Hz, A_2X_2, 4H, H-2',3',5',6'), 8.05 (dd, $J_{7,6}$ = 8 Hz, $J_{4,7}$ = 0.8 Hz, 1H, H-7), 8.15-8.35 (m, 3H, H-4,6 and N^1-H). - MS (70 eV) m/z: 325 (100 %, M^+), 310 (12), 296 (13), 280 (78), 234 (12), 207 (10). - $C_{17}H_{16}O_4N_3Cl$ (361.5) Calcd. C 56.4 H 4.43 N 11.6 Found C 56.1 H 4.38 N 11.3

2-(Phenoxy)methyl-5-[1H]benzimidazole carboxylic acid ethyl ester HCl (**7a**)

7a was obtained from **7** and esterification of compound **9**, by using dry HCl and absol. EtOH, as white powder, m.p 242°C, yield 44 %. - ^{1}H-NMR: 1.40 (t, J = 7 Hz, 3H, CH_2CH_3), 4.40 (q, J = 7 Hz, 2H, CH_2CH_3), 5.70 (s, 2H, CH_2), 6.9-7.5 (m, 5H, H-phenyl), 7.9 (d, $J_{7,6}$ = 8 Hz, 1H, H-7), 8.1 (dd, $J_{6,7}$ = 8 Hz, $J_{4,6}$ = 1.45 Hz, 2H, H-4,6), 8.30 (broad s, N^+-H), 8.4 (s, 1H, N^1-H). - MS (70 eV) m/z: 296 (7 %, M^+), 203 (100), 189 (14), 174 (29), 158 (13), 131 (29), 65 (10). - $C_{17}H_{17}O_3N_2Cl$ (332.5) Calcd. C 61.35 H 5.11 N 8.42 Found C 61.0 H 5.05 N 8.10.

2-(4-Chlorophenoxy)methyl-5-[1H]benzimidazole carboxylic acid ethyl ester HCl (**8a**)

8a was obtained from **8** as white powder, m.p 267°C, yield 41 %. - ^{1}H-NMR: 1.40 (t, J = 7 Hz, 3H, CH_2CH_3), 4.40 (q, J = 7 Hz, 2H, CH_2CH_3), 5.70 (s, 2H, CH_2), 6.25 (braod s, N^+-H), 7.2 and 7.4 (2d, $J_{2',3'}$ and $J_{5',6'}$ = 10 Hz, A_2X_2, 4H, H-2',3',5',6'), 7.8 (d, $J_{7,6}$ = 8 Hz, 1H, H-7), 8.1 (dd, $J_{6,7}$ = 8 Hz, $J_{4,6}$ = 1.45 Hz, 2H, H-4,6), 8.30 (s, 1H, N^1-H). - MS (70 eV) m/z: 332 (3 %, M^+), 330 (10, M^+), 287 (1.5), 285 (5), 203 (100), 175 (41), 158 (11), 132 (30). - $C_{17}H_{16}O_3N_2Cl_2$ (367). - Calcd. C 55.6 H 4.36 N 7.63 Found C 55.9 H 4.72 N 7.67.

2-(Phenoxy)methyl-5-[1H]benzimidazole carboxylic acid (**9**)

Ethyl 3,4-diaminobenzoat (0.905 g, 5 mmol), phenoxyacetic acid (0.76 g, 5 mmol) and 4N HCl (25 ml) were heated under refluxed for 4 h. After cooling, the mixture was adjusted to pH 7 with 10 % NaOH. The precipitated solid was collected, and washed with water. After drying, the crude product was purified on a column of silicagel (0.06-0.20 mm diameter) with $CHCl_3$: isopropanol (8:2), m.p 135°C, Yield 19.8, IR cm^{-1}: 1690 (COOH), ^{1}H-NMR: 5.40 (s, 2H, CH_2), 6.9-7.5 (m, 5H, H-phenyl), 7.6 (d, $J_{7,6}$ = 8 Hz, 1H, H-7), 7.9 (dd, $J_{6,7}$ = 8 Hz, $J_{4,6}$ = 1.43 Hz, 2H, H-4,6), 8.30 (s, 1H, N^1-H). - MS (70 eV) m/z: 268 (14 %, M^+), 174 (100), 130 (10). - $C_{15}H_{12}O_3N_2$ (268) Calcd. C 67.16 H 4.48 N 10.45 Found C 67.5 H 4.18 N 10.75

References

1 D.W. Woolley, J. Biol. Chem. *152*, 225 (1944).
2 H.P.R. Seeliger, Mykosen *1*, 1 (1958).

3 D. Berg, K.-H. Büchel, M. Plembel, and A. Zywietz, Mykosen *29*, 221 (1986); C.A. *105*, 34969z (1986)
4 L.C. Davidse and W. Flach, Biochim. Biophys. Acta *543*, 82 (1978).
5 M.A. Phillips, J. Chem. Soc. *1928*, 2395.
6 G. Anderson, Testing of Susceptibility to Antimicrobial Agents in Body Fluids. In: J.E. Blair, E.H, Lennette, J.P. Truant: Manual of Clinical Microbiology. Williams-Wilkins company, Baltimore, Maryland 1970.
7 E. Ritsert and W. Epstein, Ger. Pat. 151,725; Chem. Zentr. *1904*, 1587.
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