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Activity of Selected 2,4-Diaminoquinazolines against *Candida albicans in Vitro*[1] (39516)

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Several years ago a broad spectrum *in vitro* antimicrobial screening program was conducted in these laboratories employing a wide variety of quinazoline derivatives (1). One compound, 6-(3,4-dichlorophenylacetamido)-2,4-diaminoquinazoline (structure A, Table I) was found to display selective inhibitory effects against *Candida albicans* in this qualitative test system. Interestingly, this compound was originally synthesized as a potential antimalarial agent by Hynes and Ashton (2). It was found to be inactive against *Plasmodium berghei* in mice as well as *Plasmodium gallinaceum* in chicks and also to be nontoxic to both host species. Based upon this information, it was suggested that A may be effective against other fungi such as *Cryptococcus neoformans*. It is well known that the chemotherapy of systemic infections due to this organism is far from satisfactory since only amphotericin B and 5-fluorocytosine are of value, and each of these drugs has major limitations (3). A recent communication by Hariri and Larsh confirmed this hypothesis since A was highly effective against *C. neoformans* in mice (3). Unfortunately, the source of the earlier results from our laboratories was inadvertently omitted from their paper. The current study was conducted to ascertain the structure-activity patterns for compounds related to compound A against *C. albicans*.

Materials and methods. The 2,4-diaminoquinazolines (compounds A–I) were available by virtue of a recent synthetic study (2). 5-Fluorocytosine was obtained from Roche Laboratories. Incubation flasks for *C. albicans* were standard 50-ml Erlenmeyer flasks with uniform-bore 19-mm-o.d. tubes fused near the tops. Tilting of the contents of each flask permitted measurement of the optical density (OD) of the contents. The strict linearity of the OD as a function of cell population was confirmed by appropriate dilutions of a late log phase culture followed by OD measurements; no attempt was made in this preliminary study to differentiate a fungistatic effect from a fungicial effect. Each flask contained 19.8 ml of sterile Sabouraud dextrose broth (Difco) and 0.2 ml of dimethylsulfoxide (DMSO) containing varying concentrations of each quinazoline compound to yield the desired final concentrations. Control flasks contained DMSO at the same final concentration. 5-Fluorocytosine was dissolved in sterile water. Inoculum was 0.2 ml of an overnight culture of *C. albicans* in Sabouraud broth. After 8 and 24 hr of incubation at 37° on a Dubnoff reciprocating shaker, the OD of each culture was measured at 640 nm with a Coleman spectrophotometer, and the concentration of each compound which conferred 50% inhibition of growth (IC_{50}) was determined graphically. Acute toxicity tests of compounds A and D were done in Sch:ARS HA(ICR)$_f$ mice (Sprague Dawley). A saline suspension of each compound was treated for 15 sec with a Branson Sonifier to yield a fine suspension. Groups of six mice received 25, 100, or 250 mk/kg of each compound ip; the concentration was adjusted so that each mouse received 1.0 ml of suspension per 30 g of body weight. Mean weights of all groups were recorded daily.

Results and discussion. As shown in Table I, several different structural modifications

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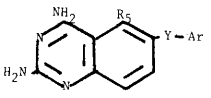
of A were evaluated for activity against *C. albicans;* results obtained with 5-fluorocytosine are included for comparison. Modifications included alteration of the aryl group (E, F, and G), modification of the bridge connecting the aryl moiety to the 6-position of the quinazoline nucleus (B, C, and D), and the insertion of a methyl or chloro group at position 5 (H and I). For the compounds studied, it is apparent that the 3,4-dichlorophenyl group affords optimal activity. Second, the insertion of a small hydrophopic group at the 5-position is deleterious to activity. Finally, the elongation of the spacer group by a methylene group (C) or its inversion (B) leads to substantial losses in potency with respect to A. However, the excision of one methylene unit (D) results in a compound significantly more active than A and which rivals 5-fluorocytosine in effectiveness.

Based upon these results, compounds A and D were selected for acute toxicity studies and the results are summarized in Table II. It will be seen that A caused no fatalities even at 250 mg/kg. On the other hand, D produced significant weight losses at 100 mg/kg and at the highest dose tested one (of six) animals failed to survive. In spite of its close structural similarity to known inhibitors of dihydrofolate reductase, compound D was found to be an exceptionally poor inhibitor of the rat liver enzyme, while A was reasonably effective (2). Therefore, it

TABLE I. *In Vitro* ACTIVITY OF 6-SUBSTITUTED-2,4-DIAMINOQUINAZOLINES AGAINST *Candida albicans*.



Compound designation	R_5	Y	Ar	IC_{50} (μg/ml) 8-Hr growth Isolate No. I	II	III	24-Hr growth Isolate No. I	II	III
A	H	$NHC(=O)CH_2$	$3,4\text{-}Cl_2C_6H_3$	0.7	3.8	2.3	2.5	5.3	3.3
B	H	$CH_2NHC(=O)$	$3,4\text{-}Cl_2C_6H_3$	5.8	8.0	>15.0	12.8	14.5	9.5
C	H	$CH_2NHC(=O)CH_2$	$3,4\text{-}Cl_2C_6H_3$	6.5	13.5	>15.0	>15.0	>15.0	>15.0
D	H	$NHC(=O)$	$3,4\text{-}Cl_2C_6H_3$	0.8	0.6	0.6	2.0	3.6	1.0
E	H	$NHC(=O)CH_2$	$3\text{-}(CF_3)C_6H_4$	6.3	15.0	8.4	11.8	>15.0	9.8
F	H	$NHC(=O)CH_2$	$4\text{-}ClC_6H_4$	3.5	4.5	>15.0	10.5	10.8	6.8
G	H	$NHC(=O)CH_2$	$2\text{-}C_{10}H_7$	3.0	2.8	13.8	6.8	10.0	4.6
H	Cl	$NHC(=O)CH_2$	$3,4\text{-}Cl_2C_6H_3$	7.0	7.3	5.4	14.3	>15.0	13.5
I	CH_3	$NHC(=O)CH_2$	$3,4\text{-}Cl_2C_6H_3$	5.6	7.4	>15.0	12.5	13.8	12.5
5-Fluorocytosine				0.5	0.4	1.2	2.7	0.7	3.0

TABLE II. ACUTE TOXICITY OF SELECTED QUINAZOLINE DERIVATIVES IN MICE.

Compound	Dose (mg/mg)	Average weight (g)[a] Days postinjection								
		0	1	2	3	4	5	6	7	8
A	25	26.9	27.3	27.4	27.7	27.8	–	28.5	28.5	28.3
A	100	27.1	25.8	26.7	27.3	27.8	–	28.6	29.0	28.9
A	250	26.0	23.8	25.3	25.8	26.0	–	27.6	27.5	27.2
D	25	27.8	25.3	24.7	25.3	26.3	–	27.5	27.7	27.2
D	100	29.9	27.8	24.8	25.8	26.7	–	26.8	26.5	26.8
D	250	27.6	25.8	23.3	21.7	21.5	–	21.6[b]	21.8[b]	22.0[b]

[a] Six mice per group.
[b] One mouse died; average weight of five remaining mice.

appears that the toxicity of D is not related to the inhibition of this metabolic transformation. In any event, neither A nor D displayed toxicity at potentially useful therapeutic levels.

In view of these results, *in vivo* studies have been initiated with these two compounds against a variety of pathogenic fungi. In addition, new structures will be synthesized in an effort to achieve higher levels of antifungal activity.

Summary. A series of nine 2,4-diaminoquinazolines bearing a variety of substituents at positions 5 and 6 was evaluated against three human isolates of *Candida albicans in vitro*. An established drug, 5-fluorocytosine, was chosen as a standard of comparison and each isolate was found to be sensitive to this agent. The two most active compounds, 6-(3,4-dichlorophenylacetamido)-2,4-diaminoquinazoline and 6-(3,4-dichlorobenzamido)-2,4-diaminoquinazoline, were selected for acute toxicity studies in mice. At the highest dose tested (250 mg/kg) the latter compound showed evidence of toxicity while both compounds were substantially free of untoward effects at lower doses.

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