Antifungal Activity of Some Substituted Pyrimidines

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Abstract: Several 6-chloro-5-nitropyrimidines were synthesized, and their antifungal activity was tested. Some derivatives were equipotent or superior to amphotericin B *in vitro* but their relatively high toxicity in mice and their chemical instability discourage further evaluation.

In the course of routine evaluation in our laboratories of various substances, both natural and synthetic, for general antimicrobial activity, it was observed that certain nitropyrimidine intermediates showed good antifungal activity. As antifungal chemotherapy as well as various agricultural practices are limited by the lack of availability of highly active antifungals that are non-toxic to the host (1), we chose to evaluate these pyrimidines in detail.

Materials and Methods

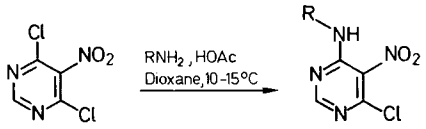
Qualitative Antimicrobial Screening – All compounds were tested for activity against the following microorganisms: *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 15442), *Mycobacterium smegmatis* (ATCC 607), *Candida albicans* (ATCC 10231), *Saccharomyces cerevisiae* (ATCC 9763), and *Aspergillus niger* (ATCC 16888). Routine qualitative screening of compounds for antimicrobial activity was accomplished as previously described (2) except for the following modifications: plates for the assay were prepared by dispensing 25 ml of sterile agar medium into 100 x 15-mm sterile petri dishes; with the use of the quadrant streak method, the sterile agar plates were streaked with a dilution of the test organism (1 ml of broth culture in 9 ml of sterile water).

Antimicrobial activity was recorded as the width (in millimeters) of the inhibition zone measured from the edge of the agar well to the edge of the inhibition zone. Zones of greater than 1 mm width are readily measured. Three replications were done.

Quantitative Antimicrobial Assay – For compounds that showed significant activity in the qualitative screen, the MIC values were determined with the two-fold serial dilution technique previously described (2). All compounds were initially tested at a concentration of 100 μg/ml in the first tube.

The MIC was taken as the lowest concentration that inhibited growth after 24 or 48 h of incubation. Tubes inoculated with fungi and yeasts were incubated at 30° for 48 h. Amphotericin B (Calbiochem, San Diego, CA) was used as a standard antibiotic for comparison with the compounds.

Synthesis of Compounds:



1 **2**

R = H **(2a)**
$CH_2CH=CH_2$ **(2b)**
$CH_2(CH_2)_3CH_3$ **(2c)**
$CH_2-C_6H_5$ **(2d)**

Scheme I

4,6 Dichloro-5-nitropyrimidine (**1**) was obtained as described by Hull (3), Boon et al. (4) and Brown (5) in 53 % overall yield. Purification of the final product was accomplished by soxlet extraction of the crude product with hexane. mp: 219–220° C (lit. mp. 220° C (5)). ^{1}H-NMR δ ($CDCl_3$): 9.0 (1H, s); Ms M^+: 196, M^++ 2: 198 (65 % M^+) M^++ 4: 200 (10 % M^+).

General Procedure for Synthesis of 4-Amino-, 4-Alkylamino-, or 4-Arylamino-6-chloro-5-nitropyrimidines: These were obtained by the procedure described by Rose (6) (Scheme I); to 7.0 mmole of the appropriate amine in H_2O (~ 5 ml) was added glacial acetic acid, enough to bring the mixture to pH 7.5–8. This solution was then added dropwise to a cooled (10–15°C) solution of **1** (0.194 g, 1 mmole) in dioxane (15 ml). After the addition was completed, the mixture was stirred for 2 h and then poured into 50 ml ice and water and extracted with chloroform. The chloroform layer was then dried with Na_2SO_4, decanted and evaporated at reduced pressure. The residue consisted of a mixture of starting material (**1**), mono, and diaminated pyrimidine. Column chromatography on silica gel 60, with chloroform as the eluent, readily separated the materials; the order of elution being starting material first, monoamino-pyrimidine second and diamino-pyrimidine last. All compounds gave satisfactory analytical and spectral characteristics.

4-Amino-6-chloro-5-nitropyrimidine (**2a**) obtained in 75 % yield, m.p. 154–155° C, (Lit. 155–156° C (4)).

4-Allylamino-6-chloro-5-nitropyrimidine (**2b**) in 60 % yield recrystallized from hexane-ether, mp. 46.5–47° C, IR (KBr) 3400 cm^{-1} (-NH) 1640, 990, 905 cm^{-1} (-CH=CH_2); ^{1}H-NMR δ ($CDCl_3$): 8.4 (1H, s, C_2H pyr.) 7.66 (1H, brs-NH) 6.3–5.6, 5.5–5.06 (3H, m characterstic of -CH=CH_2), 4.33 (2H, m, -CH_2-); MS *m/z*: M^+, 214, M^+ + 2, 216. (30 % M^+). Anal. Calc. $C_7H_7ClN_4O_2$: C, 39.18; H, 3.29; N, 26.10. Found: C, 39.01; H, 3.47; N, 26.01.

4-n-Pentylamino-6-chloro-5-nitropyrimidine (**2c**) in 66 % yield, light yellow oil, IR (neat) 3400 cm^{-1}(-NH-); ^{1}H-NMR δ ($CDCl_3$): 8.30 (1H, s, C_2H pyr.), 7.63 (1H, brs, -NH-), 3.58 (2H, d of t, *J*=2, 6 Hz, NH-CH_2-$(CH_2)_3$ CH_3); MS *m/z*: M^+, 244, M^+ + 2, 246. (30 % M^+). Anal. Calc. $C_9H_{13}ClN_4O_2$: C, 44.18; H, 5.35; N, 22.90. Found: C, 43.95; H, 5.01; N, 23.02.

4-Benzylamino-6-chloro-5-nitropyrimidine (**2d**) in 75 % yield m.p.: 129–130°C, IR (KBr): 3410 cm^{-1} (-NH-); ^{1}H-NMR δ ($CDCl_3$): 8.3 (1H, s, C_2H pyr.), 7.66 (1H, brs, -NH-), 7.25 (5H, s, -ArH), 4.75 (2H, d, *J*=6Hz, -CH_2-Ar); MS *m/z*: M^+, 264, M^+ + 2, 266. (30 % M^+). Anal. Calc. $C_{11}H_9ClN_4O_2$: C, 49.92; H, 3.43; N, 21.17. Found: C, 49.61; H, 3.71; N, 21.30.

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Results and Discussion

The variously substituted pyrimidines appeared to have negligible antibacterial activity against the strains of the five bacteria used in the routine screening procedures. No further antibacterial studies were pursued.

The qualitative antifungal activities using an agar well diffusion assay (Table I) of the materials were comparable to those of the standard amphotericin B under the test conditions. The minimum inhibitory concentration (MIC) of each compound was determined using a twofold serial dilution assay (Table II). All the substituted 4-amino-6-chloro-5-nitropyrimidines show activity comparable to or greater than the standard, amphotericin B. The unsubstituted analog, **2a**, was the least active and was inhibitory at concentrations of 100 μg/ml for *Saccharomyces cerevisiae* and *Candida albicans* and inhibitory for *Asperigillus niger* only at concentrations greater than 100 μg/ml. The benzyl derivative, **2d**, was the least active of the substituted compounds, showing inhibition of *Saccharomyces cerevisiae* and *Candida albicans* at concentrations of 50 μg/ml or greater. Like the unsubstituted material **2a**, it was inhibitory for *Aspergillus niger* only at concentrations greater than 100 μg/ml. Substitution of the 4 amine group with an allyl, yielding **2b**, enhances activity against both *Saccharomyces cerevisiae* and *Candida albicans* but not against *Aspergillus niger*. Substitution of the 4-amine group with a saturated alkyl group, the *n*-pentyl, **2c**, enhances activity against all three fungal organisms. Compound **2c** is the most active compound of the series.

Table I. Antifungal Activity of Various 6-Chloro-5-nitropyrimidines

Compound[a]	Radius of Zone of Inhibition, mm *Saccharomyces cerevisiae*	*Candida albicans*	*Aspergillus niger*
1	4	4	–
2a	3	–	–
2b	4	2	–
2c	5	6	4
2d	4	5	2
Amphotericin B	11	10	6

[a]All test compounds were tested at a concentration of 1 mg/ml in dimethyl sulfoxide.

Table II. Minimum Inhibitory Concentrations (Micrograms per Milliliter) of Various 6-Chloro-5-nitropyrimidines

Compound	*Saccharomyces cerevisiae*	*Candida albicans*	*Aspergillus niger*
2a	100	100	>100
2b	6.3	25	100
2c	3.1	25	50
2d	50	50	>100
Amphotericin B	25	25	50

Gershon and Parmegiani have reported that poly-chlorinated pyrimidines possess antifungal activity (7). They noted that at least the trichlorinated derivatives were required for good activity. In our case only a single chlorine is present, but its reactivity is enhanced by the adjacent nitro group. The increased activity seen in the pyrimidines reported herein is likely due to a combination of the high reactivity of the 6-chloro group and the enhancing effect of the 4-alkylamino group on solubility and absorption into the fungi.

Compounds **2b** and **2d** were evaluated for their toxicity in mice. They proved moderately toxic (LD_{50}'s 50–60 mg/kg) and were CNS depressant, which makes them somewhat more toxic than the standard antifungal amphotericin B (LD_{50}88 mg/kg) in mice (8). In addition, when these materials were dissolved in hydroxylic solvents, stored (even at low temperatures) for more than 2 days and then tested for their antifungal activity, they were no longer inhibitory to fungal growth. Chromatographic and spectroscopic evaluation of the residues obtained by evaporation of the solvent showed that the substances had undergone extensive degradation. Although somewhat more stable in non-hydroxylic solvents, the substances also degraded in all solutions, and samples of **2c** stored neat lost activity after some weeks. Amphotericin B solutions prepared and stored similarly still retain their activity after a few days. This lack of stability and the toxicity of these compounds against mice, have discouraged us from further evaluation of the series.

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