Anticoccidial Activity of 1-Phenyluracils[1]

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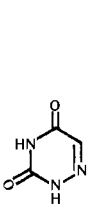
The minimum effective concentration (MEC) of 6-azauracil against *Eimeria tenella* when incorporated in feed is about 1000 ppm. Attachment of suitably substituted phenyl side chains at the 1-position reduces the MEC to less then 1 ppm. Uracil itself is devoid of anticoccidial activity, but side chains attached at the 1-position potentiate uracil and result in activity against *E. tenella*. Although effective at levels as low as 60 ppm, the 1-phenyluracils are less active than the corresponding 1-phenyl-6-azauracils. They also are less acidic.

Although 6-azauracil (1) is effective against *Eimeria*



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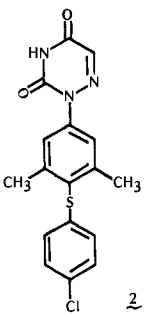

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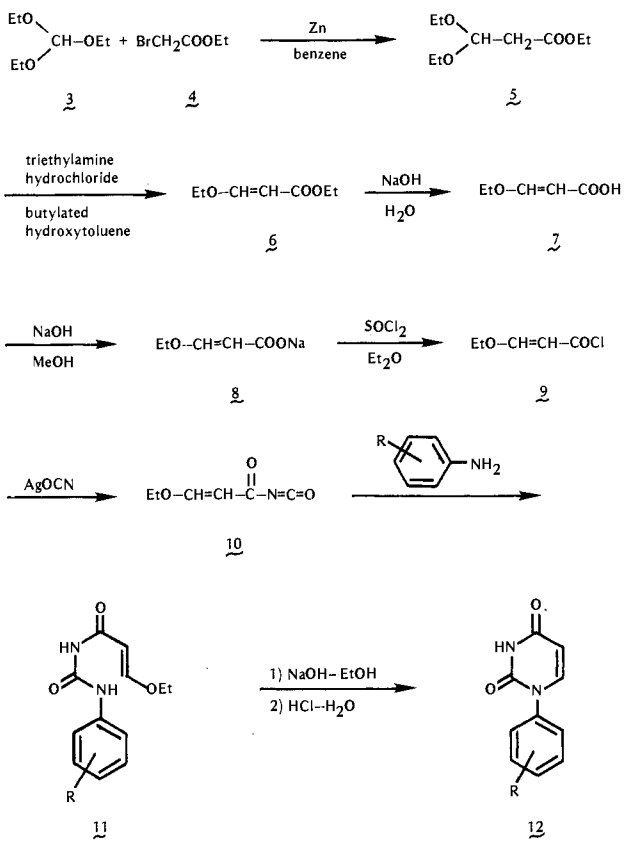
tenella at a concentration of 1000 ppm in the feed, attachment of suitably substituted phenyl side chains at the 1-position yields compounds effective at less than 1 ppm. Tiazuril (2), for example, prevents infections at 0.5 ppm or about 500 mg/ton of feed.[3]

It is believed that 6-azauracil is converted in vivo to its nucleotide, which inhibits orotidylic acid decarboxylase, an enzyme in the biosynthetic pathway to the nucleic acid pyrimidines.[3] Lately, additional modes of action of 6-azauracil have been identified in certain biological systems.[4] The inhibition of inosinate dehydrogenase by 6-azauridine has been reported.[5,6]

We found that replacement of the ribose phosphate moiety of 6-azauridylic acid with phenyl side chains as in 2 increases the lipophilicity of the compounds, as well as the acidity of the imide proton. These changes, combined with certain steric constraints, contribute to the increased activity against the protozoa. That is, the 1-phenyl-6-azauracils probably bind more tightly to the pertinent enzymes of the coccidia than does the natural substrate.[3,7-13]

Uracil, which also is converted to the nucleotide in vivo, has no activity against *E. tenella*. We thought that it would be interesting to see if the same side chains that potentiated 6-azauracil would confer anticoccidial activity on uracil.

Scheme I



Results and Discussion

No direct synthesis of 1-phenyluracils was available. An adaptation of a combination of published methods[14-16] was arrived at which allowed the use of anilines for the introduction of the side chain. This permitted the application of excess side-chain anilines on hand from the 6-azauracil work. The general procedure is shown in Scheme I. We found that practical yields of 6 were obtained only if a polymerization inhibitor, such as butylated hydroxytoluene, were added to the mixture prior to heating. Attachment of suitably substituted phenyl side chains at the 1-position of uracil did, indeed, confer a significant degree of activity against *E. tenella*. The physical and biological properties of the compounds prepared are summarized in Table I. Generally, the more complex side chains that

(1) The results reported here are included also in M. W. Miller, U.S. Patent 4 239 888 (1980).
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(3) M. W. Miller, B. L. Mylari, H. L. Howes, Jr., S. K. Figdor, M. J. Lynch, J. E. Lynch, S. K. Gupta, L. R. Chappel, and R. C. Koch, *J. Med. Chem.*, **24**, 1337 (1981).
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(16) G. Shaw and R. N. Warrener, *J. Chem. Soc.*, 157 (1958).
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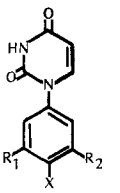
Table I. Substituted 1-Phenyl-2,4(1*H*,3*H*)-pyrimidinediones (1-Phenyluracils) and Their Activity against *E. tenella*



no.	R_1	R_2	X	mol formula	anal.[a]	mp, °C	MEC,[a] ppm
13	Cl	Cl	H	$C_{10}H_6Cl_2N_2O_2$	C, H, N	235	125
14	Me	Me	H	$C_{12}H_{12}N_2O_2$	C, H, N	173-174	>250
15	Cl	H	*p*-Cl-C_6H_4O	$C_{16}H_{10}Cl_2N_2O_3$	C, H, N	260-262	>500
16	Cl	Me	*p*-Cl-C_6H_4-S	$C_{17}H_{12}Cl_2N_2O_2S$	C, H, N	220-227	60
17	Cl	Me	*p*-Cl-C_6H_4-SO_2	$C_{17}H_{12}Cl_2N_2O_4S$	*b*	247-248	<<125
18	Me	Me	*p*-Cl-C_6H_4-S	$C_{18}H_{15}ClN_2O_2S$	*c*	212-213	500

[a] Minimum concentrations (parts per million in feed) effective in preventing infections and maintaining zero lesion scores. [b] Anal. Calcd for $C_{17}H_{12}Cl_2N_2O_4S$: C, 49.57; H, 2.92; N, 6.80. Found: C, 49.12; H, 2.50; N, 6.34. [c] Anal. Calcd for $C_{18}H_{15}ClN_2O_2S$: C, 60.17; H, 4.18; N, 7.80. Found: C, 59.60; H, 3.86; N, 7.39.

conferred the highest potency on 6-azauracil did the same for uracil. There were interesting differences, however. It seemed that there was greater sensitivity of the biological activity to subtle structural changes than in the 6-azauracil series. Thus, **16** differs from **18** only in the replacement of Me by Cl, yet it is 10-fold more potent. In the 6-azauracil series, the difference was just twofold.[19]

In the 6-azauracils, the acidity of the imide proton, often reflecting the electronic character of substituents on the phenyl side chains, was thought to be an important factor contributing to potency. A comparison of the acidity of **13** with the acidity of the corresponding 6-azauracil (compound 14, ref 3), $pH_{1/2}$ = 10.27 vs. $pH_{1/2}$ = 7.59, showed the uracils, as anticipated, to be less acidic than the 6-azauracils.[20] One explanation for the higher potency of the 6-azauracils might be that their higher acidity promotes binding to the pertinent enzyme. Lessening the dominance of this feature in the uracil series may accentuate the importance of lipophilicity, steric nature, or other structural features, thus accounting for the wider swings in biological effects after subtle structural changes. It is noteworthy that 1-phenyl-5-azauracils also show significant anticoccidial activity.[21]

Experimental Section

Melting points were determined on a calibrated Kofler hot-stage microscope. Solvents used were analytical reagent grade and, where pertinent, were protected from water by storage over molecular sieves. Mass spectra were obtained with a Hitachi Model RMU-6E mass spectrograph. Nuclear magnetic resonance spectra were obtained for selected compounds with a Perkin-Elmer/Hitachi Model R-20 spectrometer. Potentiometric titrations were performed with a Metrohm Potentiograph E436. Thin-layer chromatography was performed with Uniplate (Analtech) precoated TLC plates (silica gel GF, 250 μm) in a variety of solvent systems.

Ethyl 3-Ethoxy-2-propenoate (6). A combination of 98 g (0.51 mol) of ethyl 3,3-diethoxypropionate (**5**), prepared in the manner described by Deno,[14] 1 g of butylated hydroxytoluene (BHT), and 1 g of triethylamine (TEA) hydrochloride was heated under N_2 at 225–230 °C in a flask equipped with a distillation head and an ebullition tube to introduce N_2. Some volatiles were collected at 60–80 °C. At intervals, the mixture was cooled, and additional 1-g increments of BHT and TEA·HCl were added, followed by more heating at 225–230 °C. When the theoretical amount of EtOH (23 g) had been collected, the mixture was distilled under vacuum, and the product was collected at 96–98 °C (16 mm) to yield 69 g (93%): n^{27}_D 1.446.

3-Ethoxy-2-propenoyl Chloride (9). This compound was prepared from **6** in the manner described by Shaw and Warrener[15] by way of 3-ethoxy-3-propenoic acid (**7**) and its sodium salt (**8**).

Phenyluracil Derivatives (13–18). These were prepared from **9** in the manner described by Shaw and Warrener[16] by way of 3-ethoxy-2-propenoyl isocyanate (**10**) and its reaction products (ureas) (**11**) with the substituted anilines. Preparation of the anilines was described elsewhere.[3,9,22]

Pharmacological Method. Testing for activity against *E. tenella* in Leghorn cockerels was done by the published method[17,18] used for the 1-substituted 6-azauracils.

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Registry No. **5**, 10601-80-6; **6**, 1001-26-9; **9**, 6191-99-7; **10**, 57796-78-8; **13**, 76993-11-8; **14**, 76993-06-1; **15**, 58290-73-6; **16**, 76993-09-4; **17**, 76993-10-7; **18**, 58290-84-9.

(19) See compounds **45** and **46** in ref 3.
(20) The $pH_{1/2}$ values were determined by potentiometric titration in 2:1 dimethylformamide/water solution.
(21) For example, see A. Haberkorn and H. P. Schulz, *Zentralbl. Bakteriol., Mikrobiol. Hyg., Abt. 1, Orig., A*, **250**, 260 (1981).
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