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Tricyclic Aryl-Substituted Anticoccidial Azauracils[1]

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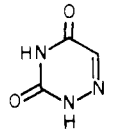
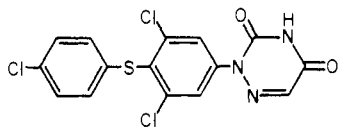
Syntheses of tricyclic aryl-substituted 6-azauracils are described. These compounds showed anticoccidial activity when tested against *Eimeria tenella* and *E. necatrix*. Compound activity was correlated with the chemical shift of the azauracil ring proton. No correlation existed between activity and compound lipophilicity. One of the compounds, 2-(11-oxo-6,11-dihydrodibenzo[*b*,*e*]thiepin-3-yl)-*as*-triazine-3,5(2*H*,4*H*)-dione (**23**), was tested extensively against *E. tenella* and *E. brunetti* both in vivo and in vitro. Compound **23** controlled mortality due to *E. tenella* at 62 ppm, and it afforded protection as measured by weight gain at 31 ppm. Compound **23** afforded little protection against *E. brunetti*. In vitro experiments with **23** showed that it exerted a coccidiostatic effect.

Weak anticoccidial activity (500 ppm) has been reported for 6-azauracil (1).[2,3] Further modification of 6-azauracil resulted in a series of 1-benzyl compounds **2**.[4] The most



1



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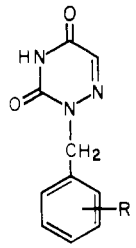

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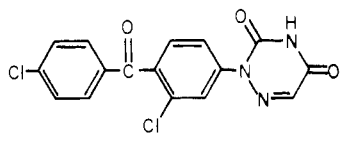

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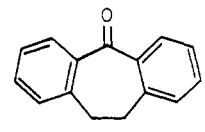
active compounds of this series were substituted with electron-withdrawing substituents in the meta position of the phenyl ring. Azauracils substituted in the 1 position with an aryl substituent were claimed in the patent literature and one of the most active of these compounds was the diphenylthioether-substituted derivative **3**.[5] A detailed report of the anticoccidial activity of **3** against *Eimeria tenella*, *E. necatrix*, and *E. brunetti* has been published.[6] The benzophenone-substituted derivative **4** has been reported effective against major *Eimeria* species at 15 ppm.[7]

The increased activity of **3** and **4** led us to attempt to combine common structural features from these molecules and to incorporate these features into other modified azauracils. A feature common to the aryl moiety of **2–4** is the presence of a meta substituent. Furthermore, there is present in the aryl moiety of **3** and **4** an ortho effect arising from 3,4-disubstitution. This buttressing effect is expected to produce a distortion from planarity of the two aryl rings and, hence, an alteration of the normal electronic contribution of the 4-substituent. Yet another property associated with nonplanarity is the alteration of the normal lipophilicity contributions associated with individual substituents.[8]

By bridging the two phenyl rings found in the azauracil substituent of **3** and **4**, one may produce a variety of tricyclic azauracil substituents which could in theory exhibit all of the substituent properties detailed above. Accordingly, the tricyclic systems **5–8** could serve as interesting substituents for azauracil modification. An important feature in tricyclics **5–8** is that they are nonplanar.



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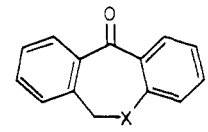
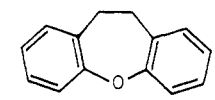


6, X = S
7, X = O



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Chemistry. The general scheme of Slouka[9,10] was modified to prepare 1-substituted 6-azauracils. This method (Scheme I) used the condensation of a diazonium salt with cyanoacetylurethane to give a hydrazone **11**, which was cyclized to a cyano-substituted azauracil **12**. The further transformation of **12** to **14** involved hydrolysis and decarboxylation steps. Since our diazonium salts were quite insoluble in water, an acetic acid–water solvent mixture was used as described in the preparation of **3**.[6] Thioglycolic acid was used to effect the decarboxylation of the azauracil-5-carboxylic acid.[11] In all cases the intermediates **11–13** were used without extensive purifica-

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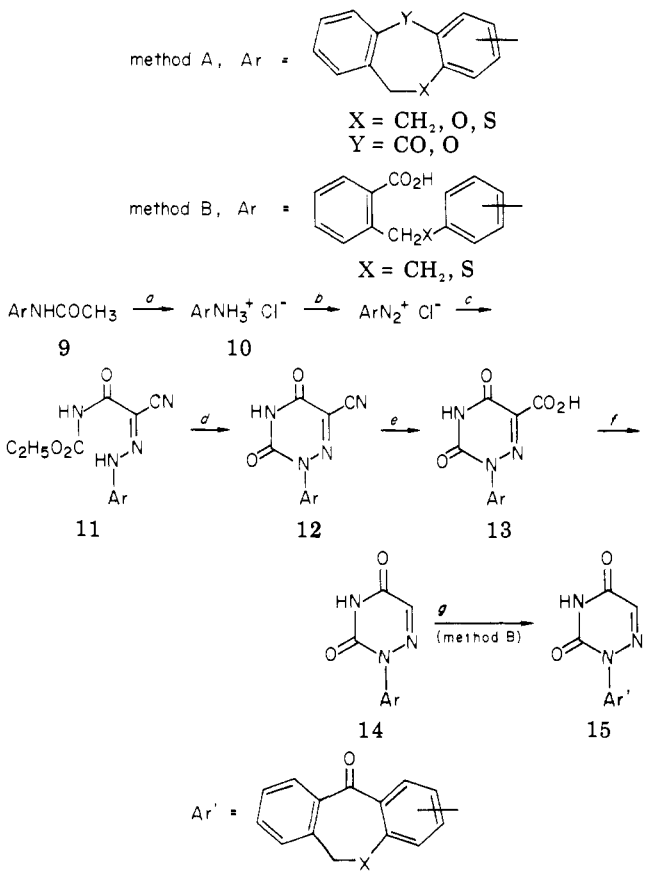
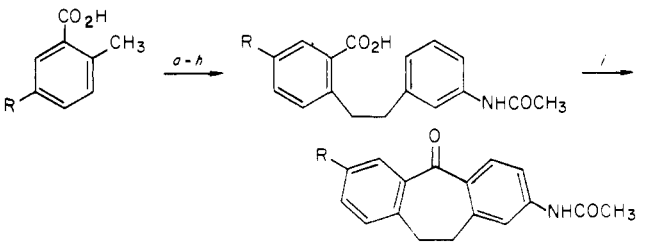
Scheme I



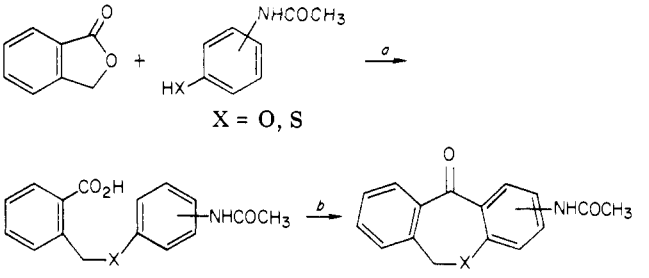
[a] HCl, H_2O. [b] HCl, $NaNO_2$, HOAc, H_2O. [c] $NCCH_2CONHCO_2C_2H_5$, K_2CO_3. [d] $(C_6H_5)_2O$, 220–250 °C. [e] HCl, H_2O. [f] $HSCH_2CO_2H$. [g] PPA, $(CH_2)_4SO_2$.

Scheme II



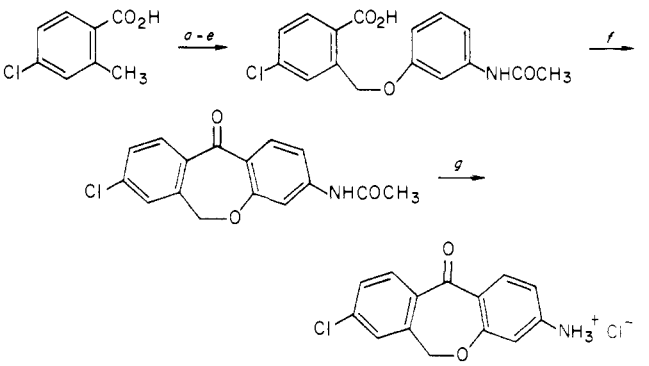
[a] CH_3I, K_2CO_3, DMF. [b] NBS, CCl_4, $h\nu$. [c] $(C_6H_5)_3P$, CH_3CN. [d] DBN, 3-$(NO_2)C_6H_4CHO$. [e] KOH, EtOH. [f] HOAc. [g] H_2, Pd/C. [h] Ac_2O. [i] PPA, $(CH_2)_4SO_2$.

Scheme III



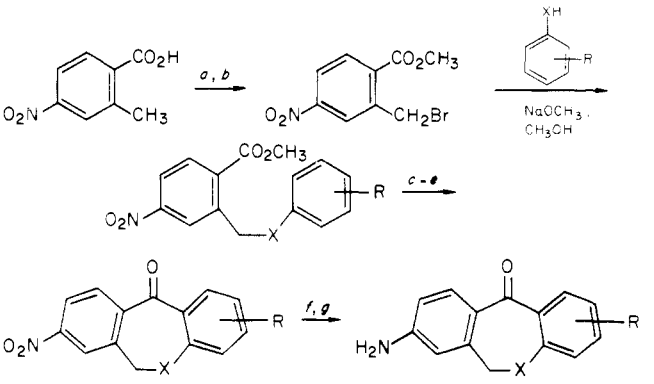
[a] NaH, DMF. [b] PPA, $(CH_2)_4SO_2$.

Scheme IV



[a] CH_3I, K_2CO_3, DMF. [b] NBS, CCl_4, $h\nu$. [c] 3-$(CH_3CONH)C_6H_4OH$, $NaOCH_3$, CH_3OH. [d] KOH, CH_3OH. [e] HCl. [f] PPA, $(CH_2)_4SO_2$. [g] HCl, H_2O.

Scheme V



[a] CH_3I, K_2CO_3, DMF. [b] NBS, CCl_4, $h\nu$. [c] KOH, CH_3OH. [d] HCl. [e] PPA, $(CH_2)_4SO_2$. [f] $SnCl_2$, HCl. [g] K_2CO_3.

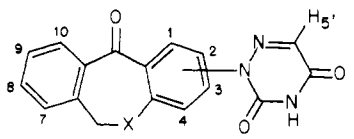
tion. The starting acetamide **9**, or the amine hydrochloride **10** (or the corresponding amine), and the final azauracil were fully characterized.

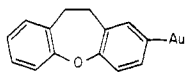
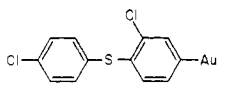
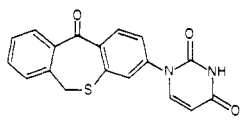
Two synthetic sequences were used. Method A involved the preparation of the tricyclic system up to the stage of an acetamide **9** or an amine **10**, whereupon the azauracil preparation proceeded according to Scheme I. In method B the aryl substituent remained uncyclized through steps a–f in Scheme I. Step g (**14** → **15**) entailed formation of the tricyclic system by polyphosphoric acid cyclization. Table I details the structures of the azauracils which were prepared by these two methods.

The acetamides **9** and amines **10** in Scheme I were prepared by modifications of literature methods. The 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one precursors were made according to Scheme II. The precursors of the 6,11-dihydrodibenz[*b*,*e*]oxepins and -thiepins were made according to Schemes III–V. The 10,11-dihydrodibenz[*b*,*f*]oxepin precursor **61** was prepared by catalytic reduction of 2-nitrodibenz[*b*,*f*]oxepin.[12] Not all of the intermediates in Schemes I–V were fully characterized. For the most part these intermediates were brought to ca. 95% purity and were carried onto the next step. Key intermediates were fully characterized. A listing of these key intermediates is found in Table II. The dibenzo[*b*,*e*]thiepin-substituted uracil **31** was prepared from the amine hydrochloride **53** according to a literature method.[13]

Since the acetamido substituent is capable of being both ortho and para directing, there is a possible ambiguity associated with the cyclizations of acids **33**, **35**, **36**, **38**, and **39** as represented in Scheme VI. A single product was

Table I. Substituted 6-Azauracils



No.	Substitution (AU = 6-azauracil) 2	3	4	8	9	X	Mol formula[a]	Mp, °C	Yield, %	Prep method	δ H_5'[b]	R_m[c]
16	H	AU	H	H	H	CH_2	$C_{18}H_{13}N_3O_3$	192-194	30[d]	A	7.62	1.05
									2[e,f]	B		
17	AU	H	H	H	H	CH_2	$C_{18}H_{13}N_3O_3$	180-181	6[e,f]	B	7.59	1.32
18	H	AU	H	H	Cl	CH_2	$C_{18}H_{12}ClN_3O_3$	254-256	10[d]	A	7.66	1.99
19	H	AU	H	H	H	O	$C_{17}H_{11}N_3O_4$	220-221	45[g]	A	7.66	0.41
20	AU	H	H	H	H	O	$C_{17}H_{11}N_3O_4$	202-205	6[d]	A	7.57	0.62
21	H	AU	H	Cl	H	O	$C_{17}H_{10}ClN_3O_4$	320	31[g]	A	7.66	*h*
22	H	H	H	AU	H	O	$C_{17}H_{11}N_3O_4$	283-285	9[g]	A	7.69	0.62
23	H	AU	H	H	H	S	$C_{17}H_{11}N_3O_3S$	229-231	25[g]	A	7.65	0.66
24	AU	H	H	H	H	S	$C_{17}H_{11}N_3O_3S$	204-205	8[d]	A	7.58	1.05
									18[e,f]	B		
25	H	H	H	AU	H	S	$C_{17}H_{11}N_3O_3S$	233-234	4[e]	A	7.64	0.99
26	H	AU	H	H	H	SO	$C_{17}H_{11}N_3O_4S$	245-247	74[i]		7.72	−0.75
27	H	H	CH_3	AU	H	S	$C_{18}H_{13}N_3O_3S$[j]	255-257	30[k]	A	7.64	1.73
28	CH_3	H	H	AU	H	S	$C_{18}H_{13}N_3O_3S$	292-294	41[l]	A	7.65	1.82
29							$C_{17}H_{13}N_3O_3$	180-181	*m*	A	7.57	1.73
30							$C_{15}H_9Cl_2N_3O_2S$[n]	175-176		A	7.61	3.48
31							$C_{18}H_{12}N_2O_3S$	>310				

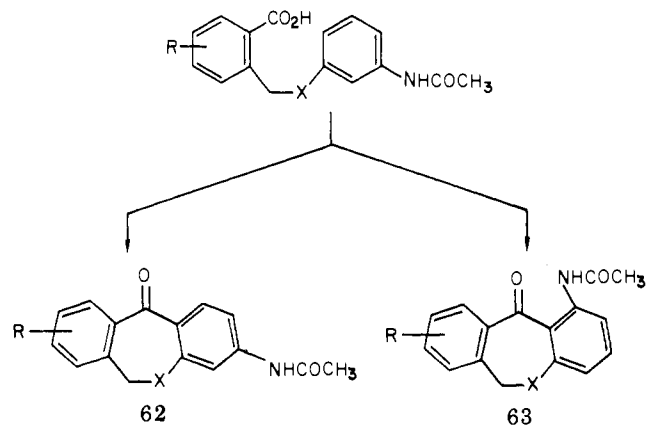
[a] Analyses for C, H, and N within ±0.4% of calculated values except where noted. [b] Ca. 5% wt/vol in Me_2SO-d_6. [c] See Experimental Section. [d] Overall yield for transformation from 9 to 14. [e] Overall yield for transformation from 10 to 15. [f] Preparative method B includes PPA cyclization to tricyclic as last step. [g] Overall yield for transformation from 10 to 14. [h] Compound insoluble in partitioning TLC solvent system. [i] Yield for oxidation from 23. [j] Anal. Calcd for $C_{18}H_{13}N_3O_3S$: C, 61.53; H, 3.73; N, 11.96. Found: C, 60.95; H, 3.86; N, 11.66. [k] Overall yield from nitro compound 59. [l] Overall yield from nitro compound 57. [m] Overall yield from 61. [n] See ref 6.

obtained from each of these PPA cyclizations, and we assign that product the structure derived from the electrophilic substitution of the carbonyl in the position which is para to the acetamido group (62). The preference for para substitution has been observed for dibenz[*b*,*f*]oxepins,[14] dibenz[*b*,*e*]oxepins,[15] and dibenzo[*b*,*e*]thiepins.[16]

NMR evidence (Table III) from the various cyclized materials (62) completely supports the structural assignment based on para substitution to the acetamido group. In all cases the proton H_1 appears as a doublet at from ca. δ 8 to 8.15 with an ortho coupling constant of from 8 to 9 Hz. If these materials have been derived from ortho substitution (63), the NMR would not be expected to exhibit a sharp doublet. The addition of shift reagent to 3-amino-6,11-dihydrodibenzo[*b*,*e*]thiepin (entry d of Table III) resolved the protons at positions 1, 2, 4, and 10. They appeared as a doublet (J_o = 9 Hz), a double doublet (J_o = 9 Hz, J_m = 2 Hz), a doublet (J_m = 2 Hz), and a broad doublet (J_o = 8 Hz), respectively. The spectrum of 3-amino-6,11-dihydrodibenz[*b*,*e*]oxepin hydrochloride (entry c in Table III) showed well-resolved absorptions for protons 1, 2, and 4: δ 6.56 (d, $J_{2,4}$ = 2 Hz), 6.75 (dd, $J_{4,2}$ = 2 Hz, $J_{1,2}$ = 9 Hz), and 8.04 (d, $J_{2,1}$ = 9 Hz).

We note also that the azauracil substituent is para directing in PPA cyclizations. Compound 16 was derived both through method A and method B. Since the position of the azauracil moiety in 16 was established from the NMR spectrum of acetamide 45, which was subsequently converted to 16, it follows that the azauracil group must be para directing in the PPA cyclization.

Scheme VI



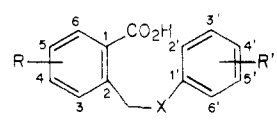
62 63

X = CH_2, O, S

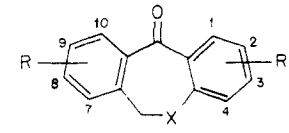
Biological Activity and Structure–Activity Relationships. Results against *E. tenella* and *E. necatrix* are recorded in Table IV. The parameters followed in compound evaluation are the anticoccidial index (ACI) and lesion scores. Table V lists anticoccidial indices found for several commercial anticoccidials.

An important substituent effect in this series is the dependence on the position of azauracil substitution relative to the carbonyl in the tricyclic. Comparison of

Table II. Key Intermediates



32-44

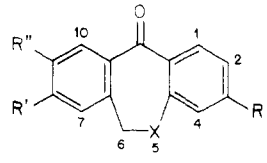


45-61

No.	X	R	R'	Mp, °C	Mol formula[a]
32	CH_2		3'-NH_2	110-110	$C_{15}H_{15}NO_2$
33	CH_2		3'-$NHCOCH_3$	143-145	$C_{17}H_{17}NO_3$
34	CH_2		4'-NH_2	147-149	$C_{15}H_{15}NO_2$
35	CH_2	5-Cl	3'-$NHCOCH_3$	122-124	$C_{17}H_{16}ClNO_3$
36	O		3'-$NHCOCH_3$	203-204	$C_{16}H_{15}NO_4$
37	O		4'-$NHCOCH_3$	234-236	$C_{16}H_{15}NO_4$
38	O	4-Cl	3'-$NHCOCH_3$	195-196	$C_{16}H_{14}ClNO_4$[b]
39	S		3'-$NHCOCH_3$	161-163	$C_{16}H_{15}NO_3S$
40	S		4'-$NHCOCH_3$	175-177	$C_{16}H_{15}NO_3S$
41	S		4'-NH_2·HCl	210-215	$C_{14}H_{14}ClNOS$
42	S	4-NO_2		144-145	$C_{14}H_{11}NO_4S$
43	S	4-NO_2	2'-CH_3	127-130	$C_{15}H_{13}NO_4S$
44	S	4-NO_2	4'-CH_3	162-164	$C_{15}H_{13}NO_4S$[c]
45	CH_2		3-$NHCOCH_3$	150-153	$C_{17}H_{15}NO_2$
46	CH_2	9-Cl	3-$NHCOCH_3$	158-159	$C_{17}H_{14}ClNO_2$
47	O		3-NH_2·HCl	165-167	$C_{14}H_{12}ClNO_2$
48	O		2-$NHCOCH_2$	155-156	$C_{16}H_{13}NO_3$
49	O	8-Cl	3-NH_2·HCl	214-216	$C_{14}H_{11}Cl_2NO_2$
50	O	8-NO_2		180-181	$C_{14}H_9NO_4$
51	O	8-NH_2·HCl		190-192	$C_{14}H_{12}ClNO_2$
52	S		3-$NHCOCH_3$	214-217	$C_{16}H_{13}NO_2S$
53	S		3-NH_2·HCl	180-185	$C_{14}H_{12}ClNOS$
54	S		2-$NHCOCH_3$	204-205	$C_{16}H_{13}NO_2S$
55	S	8-NO_2		182-184	$C_{14}H_9NO_3S$
56	S	8-NH_2		168-170	$C_{14}H_{11}NOS$
57	S	8-NO_2	2-CH_3	139-140	$C_{15}H_{11}NO_3S$
58	S	8-NH_2	2-CH_3	136-137	$C_{15}H_{13}NOS$
59	S	8-NO_2	4-CH_3	189-192	$C_{15}H_{11}NO_3S·H_2O$[c]
60	S	8-NH_2	4-CH_3	184-186	$C_{15}H_{13}NOS$
61				236-238	$C_{14}H_{14}ClNO$

[a] Analyses for C, H, and N (and Cl) within ±0.4% of calculated values except where noted. [b] Anal. Calcd for $C_{16}H_{14}ClNO_4$: C, 60.1; H, 4.41; N, 4.38; Cl, 11.09. Found: C, 59.35; H, 4.23; N, 4.15; Cl, 10.61. [c] Anal. Calcd for $C_{15}H_{11}NO_3S·H_2O$: C, 59.39; H, 4.32; N, 4.62. Found: C, 58.97; H, 5.29; N, 4.00.

Table III. NMR Data for Selected Tricyclics



Entry	X	R	R'	R''	H_1	$J_{1,2}$, Hz
a	CH_2	NHAc	H	H	8.06[a]	8
b	CH_2	NHAc	H	Cl	7.99[b]	8.5
c	O	NH_2·HCl	H	H	8.04[b]	9
d	S	NH_2	H	H	8.15[a]	9
e	S	NHAc	H	H	8.08[b]	9
f	CH_2	AU[c]	H	H	8.04[b]	9

[a] $CDCl_3$. [b] Me_2SO-d_6. [c] AU = 6-azauracil.

pairs of positional isomers (16:17, 19:20, 23:24) indicates that the more active isomer has the azauracil substituted para to the carbonyl of the tricyclic. This dependence of activity on carbonyl positioning holds for thiepin **25** but not for oxepin **22**.

The biological activity of these azauracils can be correlated in a general way with electronic effects of the tricyclic substituents. Variations in the chemical shift of the azauracil ring proton $H_{5'}$ (Table I) result primarily from variations in the electronic contributions arising from the tricyclic substituents. The average of δ $H_{5'}$ for the active "para" compounds **16**, **19**, and **23** is 7.64 ± 0.02, while the less active "meta" compounds **17**, **20**, and **24** show an average δ $H_{5'}$ of 7.58 ± 0.01. The high correlation of lesion score with δ $H_{5'}$ can be seen with the fact that seven compounds at ca. the 125-ppm dose level have lesion scores in the 0–1 range and have δ $H_{5'}$ ranging from 7.61 to 7.66. Exceptions to this correlation are **21**, **26**, and **27**.

There appears to be no consistent trend relating compound lipophilicity to activity (R_m vs. ACI). Increasing lipophilicity by chlorination gives mixed results: **18** is more active than **16**, but **21** is less active than **19**. A similar situation held for methyl substitution in that **28** was more active than **25**, but **27** was less active than the parent.

The inactivity of the uracil derivative **31** suggests a close dependence on azauracil with these tricyclic systems in order to achieve biological activity. Substituted uracils have been reported as anticoccidials.[17]

Further Study of Compound 23. In Table VI it is shown that **23** gave a slight indication of toxicity at 250 ppm in both experiments but nothing significant at 125 ppm. Compound **23** controlled mortality due to *E. tenella* at 62 ppm and gave quite a degree of control at 31 and 16 ppm. Weight gains were good down to 31 ppm and not bad at 16 ppm. Feces only showed evidence of abnormality at 16 ppm. There was, however, oocyst production at all levels tested, though suppression was almost complete at 250 ppm. Control with monensin at 100 ppm was poor, with lasalocid at 75 ppm a little better, and with Lerbek and robenidine good.

Wih *E. brunetti* by contrast, **23** showed little activity as measured by weight gains. Figures were better than controls and mortality was reduced, but the distinction was

Table IV. Anticoccidial Indices and Lesion Scores

No.	Dose, ppm[a]	ACI[b]	Lesion score[c] E.n.	Lesion score[c] E.t.
16	125	187	0-1	0
	250	179[d]	0-1	0
17	250	115	3	3
18	16	185	0-1	0-1
	31	180[d]	0-1	0
	63	192[e]	0	0
	125	193	0	0
19	16	154[d]	2-3	1-3
	31	183[e]	1-2	0-1
	63	192[e]	0-1	0
	125	189[e]	0-1	0
	250	175	0	0
20	125	0	3	3
21	8	0	3	3
	16	0	3	3
	31	84	3	3
	63	95	3	3
	125	150	3	3
22	16	97	3	3
	31	52	3	3
	63	15	3	3
	125	52	3	3
23	16	163[d]	2	1-2
	31	182[e]	1-2	0-1
	63	190[e]	0-1	0
	125	204[d]	0	0
	250	182	0	0
24	250	154	3	3
25	16	142	3	3
	31	160	2	2
	63	179	0-1	0-1
26	16	160	3	3
	31	160	3	3
	63	173	3	2
	125	167	1	1
27	16	50	3	3
	31	92	3	3
	63	127	2	2
	125	187	1-2	1
28	16	198	1-21	1-2
	31	206	0-2	0-2
	63	196	0	0
	125	209	0	0
29	16	165	3	3
	31	145	3	3
	63	165	3	3
	125	181	2	2
30	31	199	0-1	0
	63	205	0	0
	125	179	0	0
31	125	26	3	3

[a] Each dosage group is for five chicks unless otherwise noted. [b] ACI = sum of percent survival plus percent weight gain over nonmedicated, noninfected control. [c] Lesions are scored: 0 = no gross lesions; 1 = slight lesions; 2 = moderate lesions; 3 = severe lesions. [d] Average of ACI values for two groups of five chicks. [e] Average of ACI values for three groups of five chicks.

Table V. Anticoccidial Indices of Standards

Compd	Dose, ppm[a]	ACI ± SD[b,c]
Amprolium + ethopabate	125 + 4	195.6 ± 9.5 (39)
Robenidine	66	199.2 ± 9.4 (36)
Monensin	121	191.0 ± 13.4 (22)
Nequinate	20	196.3 ± 15.4 (34)

[a] Each test consisted of 20 chicks. [b] See footnote *b* in Table IV. [c] Number in parentheses refers to number of tests.

minor. Oocysts were produced at all levels and feces were abnormal. The other drugs showed a similar pattern of activity as had been observed with *E. tenella*.

Tissue Culture Studies with Compound 23. When tested for activity against *E. tenella* in vitro using chick kidney tissue, the compound at 9 ppm caused some host cell toxicity, while concentrations down to 0.11 ppm completely suppressed the development of second generation schizonts. Examination of hematoxylin- and eosin-stained preparations revealed that the compound at active levels was preventing the establishment of second generation schizonts. Mature first-generation schizonts were seen from 48 h on in treated cultures, some of which were able to release their merozoites. Some merozoites invaded host cells, but no further development was observed. Other schizonts were only able to form a small number of merozoites around a central core of nucleated cytoplasm, while others were inhibited before merozoite formation and subsequently degenerated.

When infected cultures were washed free of drug at intervals up to 48 h after inoculation, the parasites were able to resume development such that normal mature and immature second-generation schizonts could be found at 96 h as in untreated control cultures. The compound therefore only exerted a coccidiostatic effect. Full control of parasite development was achieved when medication with 0.11 ppm commenced 24 h after inoculation and partial control if medicated from 48 or 75 h onward. By increasing the concentration to 0.33 ppm, full control could be achieved with medication from 48 h on.

It was not possible to reverse the activity of the 0.1-ppm drug by the addition of 10 ppm or less of the following compounds: adenine, adenine arabinoside, adenylic acid, cytidine, cytosine, cytosine arabinoside, guanine, orotic acid, thymidine, thymine, uracil, uridine, UMP, UDP, or UTP.

The mode of action in vitro of **23** thus follows very closely that of azauracil **3**.[5,6] Azauracil **3** was, however, much more active in vitro, 0.004 ppm against *E. tenella*, but not active at 0.33 ppm, the maximum dose tolerated by the host cells, against *E. brunetti*.

Conclusions

The objective of this study was to evaluate the effect of selected tricyclic substituents on the anticoccidial activity of azauracil. It was shown that the substitution of azauracil with a dibenz[*b*,*e*]oxepin or a dibenzo[*b*,*e*]thiepin afforded compounds which were active against *E. tenella* and *E. necatrix*. None of these compounds was as active as the lead compounds **3** and **4**. Detailed study of one compound (**23**) indicated problems with control of oocyst production and with control of *E. brunetti*. These problems mean that use of **23** would require its formulation as a mixture with another anticoccidial in order to obtain wide-spectrum activity and control of oocysts. These factors indicate at best a marginal position for **23** in the anticoccidial armamentarium.

Experimental Section

In addition to combustion analysis data, the compounds in Tables I and II were examined by NMR, IR, and mass spectrometry. NMR spectra were recorded on either a Varian A-60A or a Varian HA 100. IR spectra were recorded with a Perkin-Elmer 257. The mass spectra were obtained using an Atlaswerke CH-4. Melting points were recorded on a Fisher-Johns melting point apparatus and are uncorrected. Combustion analyses were performed by the Syntex Analytical Staff and by A. Bernhardt (Mulheim-Ruhr).

Anticoccidial Challenge Assays. The data for Tables IV and V were obtained using the following experimental methods. Day-old white leghorn cockerels were placed in brooders under standard conditions. At 13 days of age the birds were weighed, were allotted five to a group in test cages, and were started on medicated diets. At day 14 the birds were inoculated with *E.*

Table VI. Further Evaluation of 23

	E. tenella (850 000 oocysts)						*E. brunetti* (168 000 oocysts)					
	% mortality	Av wt gain Days −1 to 3	Av wt gain Days 3 to 8	Faecal score	Oocysts (10^6/chick)	% sporulation	% mortality	Av wt gain Days −1 to 3	Av wt gain Days 3 to 7	Faecal score	Oocysts (10^6/chick)	% sporulation
Noninfected control	0.0	32.4	35.3	0.0	0.20	94	0.0	28.5	25.0	0.0	0.16	95
Infected control	91.7	25.8	−12.9	3.0			22.9	31.8	−24.5	2.0	5.73	94
23, 250 ppm	0.0	17.6	33.5	0.0	0.01		2.1	21.3	−3.5	1.0	0.43	90
23, 125 ppm	0.0	24.5	33.3	0.0	0.79	94	0.0	27.9	−2.9	1.0	1.47	96
23, 62 ppm	0.0	25.2	29.6	0.0	5.89	96	2.1	26.5	−3.7	1.2	3.70	94
23, 31 ppm	4.3	21.0	36.7	0.0	6.33	96	0.0	27.7	−6.5	1.7	12.70	92
23, 16 ppm	10.4	27.4	21.3	0.7	20.53	83	6.3	28.8	−14.6	2.0	9.26	84
Monensin, 100 ppm	56.3	21.5	−10.2	1.0	5.29	80	2.1	31.5	10.7	0.8	8.43	98
Robenidine, 33 ppm	0.0	23.4	33.3	0.0	0.00		0.0	29.1	27.3	0.0	0.00	
Lerbek, 108 ppm	0.0	20.6	45.2	0.0	0.00		0.0	31.3	26.0	0.0	0.00	
Lasalocid, 75 ppm	17.0	23.8	17.5	0.0	14.10	79	2.1	30.7	15.5	0.2	3.06	96

tenella plus *E. necatrix*. Seven days after infection the birds were weighed and were sacrificed. When preparing the inoculum, the aim was to produce 50% mortality and 50% body weight gain in the infected nonmedicated control birds. In 44 separate tests, with 20 birds in a control group in each test, the actual percent survivial ± standard deviation was 60 ± 26, and the percent weight gain ± standard deviation was 42 ± 17.

Compound efficacy is based on bird mortality, body weight gain, and severity of lesions. Test materials with an ACI approaching that of the standards and/or that significantly reduce lesions are considered to exhibit activity. The ACI is the sum of the percent survival and percent weight gain for a given group. For percent weight gain, all groups are compared with the nonmedicated, noninfected control group. Intestinal lesions are observed grossly and are graded as follows: 0, no obvious lesions present; 1, slight lesions; 2, moderate lesions; 3, severe lesions.

Further Study of Compound 23. Two experiments were carried out, one with *E. tenella* and one with *E. brunetti*. Each experiment utilized 768 chicks; 16 different treatments were involved, each given to six replicates of eight chicks. Birds were weighed, grouped, and put on a diet the day before inoculation, were inoculated on day 0 with 850 000 oocysts of *E. tenella* or 168 000 oocysts of *E. brunetti*, and were weighed again on day 3 and day 8 or 7, respectively, when the experiments were terminated. Birds which died were also weighed. Feces were scored visually for the presence of blood or diarrhea on day 4, and oocyst counts were done over days 5–8 or 4–7. Oocysts were extracted and put to sporulate at 30 °C in dichromate, being examined 3 days later. Weight gains over days −1 to 3 indicate possible toxicity of the treatment; weight gains over the second period are a reflection of any anticoccidial activity, complicated by toxicity where it occurs.

I. Examples of Schemes II–V. A. Scheme II. 2-Acetamido-7-chloro-10,11-dihydrodibenzo[*a*,*d*]cyclohepten-5-one (46). To a solution of 44.6 g of triphenylphosphine (0.17 mol) in 120 mL of CH_3CN was added a solution of 50.9 g of crude methyl 2-bromomethyl-5-chlorobenzoate (prepared by NBS bromination of methyl 2-methyl-5-chlorobenzoate) in 100 mL of CH_3CN. The mixture was heated at reflux for 1 h. The mixture was cooled to room temperature and was added to 700 mL of Et_2O. The resulting mixture was set aside overnight. After filtration and washing with Et_2O there was obtained 78.5 g of crude phosphonium salt which was used in the next step. To a 500-mL three-neck flask equipped with a mechanical stirrer and a reflux condenser were added 52.6 g of the above phosphonium salt (ca. 0.1 mol), 15.8 g of *m*-nitrobenzaldehyde (0.1046 mol), 250 mL of CH_3CN, and 13.2 g of diazabicyclo[4.3.0]nonene (0.106 mol). The mixture was heated at reflux for 40 min. Removal of solvent under reduced pressure left a residue which was taken up in 250 mL of $CHCl_3$. The $CHCl_3$ solution was extracted with three 100-mL portions of 5% HCl. Removal of the $CHCl_3$ under reduced pressure left a residue which was then mixed with 100 mL of CH_3OH and a solution of 25 g of KOH in 150 mL of water. The resulting mixture was heated at reflux for 18 h. The mixture was placed on a rotary evaporator to remove most of the CH_3OH. The resulting mixture was extracted with four 50-mL portions of $CHCl_3$. The aqueous layer was acidified with HCl. The precipitated acid was collected by filtration and was washed with water. This procedure gave 33.6 g of crude *cis*- and *trans*-2-carboxy-4-chloro-3′-nitrostyrene as yellow crystals, mp 175–190 °C. A mixture of 33 g of *cis*- and *trans*-2-carboxy-4-chloro-3′-nitrostyrene, 200 mL of HOAc, and 1 g of 10% Pd/C was hydrogenated at an initial pressure of 60 psi. After the calculated amount of hydrogen was absorbed the mixture was filtered and was concentrated to a volume of ca. 120 mL under reduced pressure. To this solution were added 20 mL of acetic anhydride and 1 drop of 70% perchloric acid. The mixture was set aside overnight. The mixture was concentrated under reduced pressure to a viscous oil. Addition of 400 mL of water gave a solid which was collected by filtration. Recrystallization from EtOH–H_2O gave 26.4 g of 2-[2-(3-acetamidophenyl)ethyl]-5-chlorobenzoic acid (**35**): mp 122–124 °C; NMR (Me_2SO-d_6) δ 2.12 (s, 3 H, CH_3CO), 2.6–3.5 (m, 4 H, CH_2CH_2), 6.8–7.9 (m, 7 H, aryl H), 9.83 (s, 1 H, CO_2H). Anal. ($C_{17}H_{16}ClNO_3$) C, H, N, Cl. To a 500-mL three-neck flask equipped with mechanical stirrer and gas inlet tube were added 13 g of **35** (40.9 mmol) and 80 mL of sulfolane. The flask was heated with an oil bath at 110 °C to dissolve the acid. The contents of the flask were maintained under N_2. Polyphosphoric acid (100 mL) was added and the mixture was stirred at 120 °C for 2 h. The mixture was poured into 1200 mL of water and the resulting mixture was stirred overnight. The crude product, a light-yellow solid, was collected by filtration. Recrystallization from EtOAc–hexane gave 5.5 g (45% from **35**) of 2-acetamido-7-chloro-10,11-dihydrodibenzo[*a*,*d*]cyclohepten-5-one (**46**) as yellow crystals: mp 158–159 °C; IR (KBr) 1700, 1625 cm^{-1}; NMR (Me_2SO-d_6) δ 2.13 (s, 3 H, CH_3CO), 3.1 (br s, 4 H, CH_2CH_2), 7.2–8.1 (m, 6 H, aryl H). Anal. ($C_{17}H_{14}ClNO_2$) C, H, N.

B. Scheme III. 3-Amino-6,11-dihydrodibenzo[*b*,*e*]thiepin-11-one Hydrochloride (53). A mixture of 28.8 g (0.1378 mol) of 3-*S*-acetylacetanilide,[18] 70 mL of 10% NaOH solution, and 30 mL of EtOH was heated on a steam bath for 20 min. The mixture was filtered from insoluble material and the filtrate was diluted with 100 mL of water. Acidification with HCl gave a solid which was collected by filtration. This procedure afforded 11.5 g (ca. 50%) of crude 3-acetamidothiophenol which was used without further purification in the next step. To a 100-mL flask equipped with a magnetic stirrer and a reflux condenser were added 3.05 g of a 57% mineral oil suspension of NaH (72.4 mmol) and 40 mL of DMF. To this mixture was added 11.5 g of 3-acetamidothiophenol (ca. 68.9 mmol) in small portions. After the cessation of gas evolution, 9.3 g (69.4 mmol) of phthalide was added and the mixture was heated at reflux for 2 h. The mixture was poured into 200 mL of water and the resulting mixture was extracted with two 50-mL portions of hexane. Acidification of the aqueous extract with HCl deposited a solid. Recrystallization from EtOH–H_2O gave 12.2 g (59%) of 2-(3-acetamidophenylthio)methylbenzoic acid (**39**) as off-white crystals, mp 161–163 °C. Anal. ($C_{16}H_{15}NO_3S$) C, H, N. To a 500-mL three-neck flask equipped with a gas inlet tube and mechanical stirrer were added 12 g of **39** (39.9 mmol) and 50 mL of sulfolane. The mixture was heated at 100 °C under N_2 to dissolve the acid. Polyphosphoric acid (100 mL) was added and the mixture was heated at 100 °C for 1 h, at which time TLC analysis indicated complete conversion

to a less polar material. The mixture was poured into 1000 mL of ice water and the resulting mixture was stirred overnight. The product was collected by filtration and was recrystallized from EtOH–H_2O to give 10 g (89% from **39**) of 3-acetamido-6,11-dihydrodibenzo[*b*,*e*]thiepin-11-one (**52**): mp 214–217 °C; NMR (Me_2SO-d_6) δ 2.07 (s, 3 H, CH_3CO), 4.22 (s, 2 H, CH_2S), 7.2–8.3 (m, 7 H, aryl H), 10.13 (s, 1 H, NH). Anal. ($C_{16}H_{13}NO_2S$) C, H, N. A mixture of 10 g of **52** (35.3 mmol) and 250 mL of 20% HCl was heated at reflux for 2 h. Upon cooling a solid was deposited. The solid was collected by filtration to give 7.15 g (73% from **52**) of 3-amino-6,11-dihydrodibenzo[*b*,*e*]thiepin-11-one hydrochloride (**53**), mp 180–185 °C. Anal. ($C_{14}H_{12}ClNOS$) C, H, N, Cl.

C. Scheme IV. 3-Amino-8-chloro-6,11-dihydrodibenz[*b*,*e*]oxepin-11-one Hydrochloride (49). To a solution of 1.8 g of Na (0.078 g-atom) in 1.0 mL of CH_3OH was added 11.6 g (76.8 mmol) of 3-acetamidophenol. To this solution was added 10.1 g (ca. 38.3 mmol) of methyl 2-bromomethyl-4-chlorobenzoate (prepared by NBS bromination of methyl 2-methyl-4-chlorobenzoate). The mixture was heated on a steam bath for 0.5 h and was left at room temperature overnight. The precipitate was collected by filtration to give 13.5 g of crude product, which appeared as a single spot by TLC. This solid was mixed with 120 mL of CH_3OH and a solution of 2.5 g of KOH in 40 mL of water. The mixture was heated with stirring at 60 °C for 1 h. The CH_3OH was removed under reduced pressure and 100 mL of water was added. Addition of HCl afforded a solid. Filtration gave 12.2 g (ca. 99% from the bromomethyl compound) of 2-(3-acetamidophenoxy)methyl-4-chlorobenzoic acid (**38**), mp 195–196 °C. Anal. Calcd for $C_{16}H_{14}ClNO_4$: C, 60.01; H, 4.41; N, 4.38; Cl, 11.09. Found: C, 59.35; H, 4.23; N, 4.15; Cl, 10.61. To a 500-mL three-neck flask equipped with gas inlet tube and mechanical stirrer was added a solution of 16 g (50 mmol) of **38** in 100 mL of sulfolane. The contents were maintained under N_2 and were heated to 100 °C. Polyphosphoric acid (100 mL) was added, and the mixture was stirred at 100 °C for 0.5 h. The mixture was poured into 1000 mL of ice water and the resulting mixture was stirred for 0.5 h. The product was collected by filtration and the wet cake was used directly in the next step. The wet product from the PPA cyclization was mixed with 300 mL of concentrated HCl and 40 mL of EtOH. The mixture was heated at reflux for 1 h, whereupon it was cooled in an ice bath. The product was isolated by filtration. This procedure gave 14 g (95% from **38**) of 3-amino-8-chloro-6,11-dihydrodibenz[*b*,*e*]oxepin-11-one hydrochloride (**49**) as a tan powder, mp 214–216 °C. Anal. ($C_{14}H_{11}Cl_2NO_2$) C, H, N, Cl.

D. Scheme V. 8-Amino-6,11-dihydrodibenzo[*b*,*e*]thiepin-11-one (56). To a solution of 1.28 g (0.056 g-atom) of sodium in 100 mL of EtOH was added 5.75 mL (6.18 g, 56.2 mmol) of thiophenol. To this solution was added 13.9 g of methyl 2-bromomethyl-4-nitrobenzoate (prepared by NBS bromination of methyl 2-methyl-4-nitrobenzoate) at room temperature for 18 h. Most of the solvent was removed under reduced pressure, and the residue was partitioned between 200 mL of $CHCl_3$ and 200 mL of 5% NaOH solution. The $CHCl_3$ layer was separated and the $CHCl_3$ was removed to give 16.57 g of crude, oily methyl 2-phenylthiomethyl-4-nitrobenzoate. This ester was mixed with 100 mL of CH_3OH and a solution of 3.4 g of KOH in 100 mL of water. This mixture was stirred at ca. 70 °C for 0.5 h. Most of the CH_3OH was removed by evaporation at reduced pressure. Water (100 mL) was added and the mixture was extracted twice with 50-mL portions of $CHCl_3$. The aqueous layer was acidified with HCl and the product was collected by filtration. Recrystallization from EtOH–H_2O gave 7.48 g of 2-phenylthiomethyl-4-nitrobenzoic acid (**42**), mp 144–145 °C. Anal. ($C_{14}H_{11}NO_4S$) C, H, N. To a 250-mL three-neck flask equipped with a mechanical stirrer and a gas inlet tube were added a solution of 3.95 g (13.7 mmol) of **42** in 18.5 mL of sulfolane and 35 mL of polyphosphoric acid. The mixture was heated at 110 °C for 1.5 h, and then it was poured into 500 mL of water. After stirring for 2 h the solid was collected by filtration and was washed with water. The solid was dissolved in 150 mL of Et_2O and the ether layer was extracted with two 30-mL portions of 5% NaOH solution. The Et_2O layer was dried over Na_2SO_4. After filtration and removal of solvent there was obtained 1.85 g (40%) of 8-nitro-6,11-dihydrodibenzo[*b*,*e*]thiepin-11-one (**55**): mp 182–184 °C; NMR ($CDCl_3$) δ 4.08 (s, 2 H, CH_2S), 7.15–8.25 (m, 7 H, aryl H); *m*/*e* 271 (M^+). Anal. ($C_{14}H_9NO_3S$) C, H, N. A mixture of 4.46 g (16.5 mmol) of **55** in 120 mL of HOAc was added to 10.3 g of $SnCl_2$ in 120 mL of concentrated HCl. The mixture was heated at reflux for 2 h. Upon cooling to 5 °C a solid precipitated. This material was collected by filtration and was suspended in 200 mL of 5% K_2CO_3 solution. The mixture was extracted thoroughly with $CHCl_3$. The combined $CHCl_3$ extracts were dried over Na_2SO_4. Removal of $CHCl_3$ left 3.17 g (80%) of 8-amino-6,11-dihydrodibenzo[*b*,*e*]thiepin-11-one (**56**): mp 168–170 °C; NMR ($CDCl_3$) δ 3.65 (br s, 2 H, NH_2), 3.88 (s, 2 H, CH_2S), 6.34 (d, 1 H, $J_{9,7}$ = 2.5 Hz, 7-H), 6.54 (dd, 1 H, $J_{10,9}$ = 8 Hz, $J_{7,9}$ = 2.5 Hz, 9-H), 7.15–7.4 (m, 3 H, 2-, 3-, 4-H), 7.69 (d, 1 H, $J_{9,10}$ = 8 Hz, 10-H), 8.24 (m, 1 H, 1-H). Anal. ($C_{14}H_{11}NOS$) C, H, N.

II. Azauracil Preparation. 2-(11-Oxo-6,11-dihydrodibenzo[*b*,*e*]thiepin-3-yl)-*as*-triazine-3,5(2*H*,4*H*)-dione (23). To a flask equipped with a magnetic stirrer and an alcohol thermometer were added 7 g (25.2 mmol) of amine hydrochloride **53**, 60 mL of HOAc, 20 mL of H_2O, and 5 mL of concentrated HCl. The mixture was cooled to ca. 0 °C with stirring. A solution of 1.73 g (25 mmol) of $NaNO_2$ in 20 mL of H_2O was added dropwise over ca. 15 min while the temperature was kept below 5 °C. The reaction became homogeneous ca. 10 min after the nitrite addition was complete. Alongside the diazotization flask was placed a 3000-mL three-neck flask equipped with a mechanical stirrer and a 250-mL dropping funnel. The dropping funnel was filled with saturated K_2CO_3 solution. The flask was immersed in a cooling bath. To the flask were added 11.5 g (73.7 mmol) of cyanoacetylurethane, 50 g of sodium acetate trihydrate, and 700 mL of water. The contents of the flask were cooled to ca. 0 °C. The mixture was stirred vigorously while the previously prepared diazonium salt solution was added in 0.5–1-mL portions along with dropwise addition of saturated K_2CO_3 solution. The rate of K_2CO_3 addition was adjusted to maintain the reaction mixture pH at ca. 8.5. The addition of the diazonium salt solution required ca. 0.5 h. The reaction mixture was stirred an additional 1 h at 0 °C and 2 h at room temperature. The yellow-orange solid was collected by filtration and was washed with water. The crude product was boiled with 500 mL of EtOH and filtered from the warm mixture. In this manner there was obtained 9 g of the crude yellow hydrazone. The hydrazone appeared as a single spot by TLC; however, it did not melt below 250 °C. Above 210 °C the hydrazone changed from yellow to tan. The crude hydrazone was mixed with 100 mL of diphenyl ether and the stirred mixture was heated for 0.25 h at 250 °C. The mixture was cooled and was poured into 400 mL of hexane. Filtration and washing with hexane afforded ca. 9 g of crude 2-(11-oxo-6,11-dihydrodibenzo[*b*,*e*]-thiepin-3-yl)-6-cyano-*as*-triazine-3,5(2*H*,4*H*)-dione which was carried onto the next step without further purification. The crude nitrile was mixed with 250 mL of 20% HCl and 40 mL of EtOH. The mixture was heated at reflux for 18 h. The product was collected by filtration after cooling to room temperature. The crude product was treated with 250 mL of 5% Na_2CO_3 at ca. 40 °C. The mixture was filtered and the filtrate was acidified with HCl. The resulting acid was collected by filtration, was washed with water, and was dried in vacuo at 60 °C. This procedure gave 5.88 g of crude 2-(11-oxo-6,11-dihydrodibenzo[*b*,*e*]thiepin-3-yl)-3,5(2*H*,4*H*)-dioxo-*as*-triazine-6-carboxylic acid as a tan powder which was used in the next step without further purification. To a 50-mL flask equipped with a magnetic stirrer and a reflux condenser connected to a gas collection tube were added 5.6 g of the crude dibenzothiepin-substituted azauracil-6-carboxylic acid and 10 mL of thioglycolic acid. The mixture was heated with an oil bath at 175 °C for 20 min, at which time gas evolution virtually ceased. The mixture was poured into 150 mL of water and the resulting mixture was basified with solid Na_2CO_3. The product was collected by filtration and was chromatographed on 150 g of silica gel with 5% CH_3OH–$CHCl_3$. The chromatographed product was washed with hot EtOAc and collected by filtration. This procedure gave 2.1 g (25% from **53**) of **23** as light yellow crystals: mp 229–231 °C; NMR (Me_2SO-d_6) δ 4.26 (s, 2 H, CH_2S), 7.2–7.6 (m, 6 H, 2-, 4-, 7-, 8-, 9-, 10-H), 7.65 (s, 1 H, N=CH), 8.14 (d, 1 H, 1-H), 12.2 (br s, 1 H, NH); *m*/*e* 337 (M^+). Anal. ($C_{17}H_{11}N_3O_3S$) C, H, N.

2-(11-Oxo-6,11-dihydrodibenzo[*b*,*e*]thiepin-3-yl 5-oxide)-*as*-triazine-3,5(2*H*,4*H*)-dione (26). To a stirred mixture of 0.496 g (14.7 mmol) of **23** in 35 mL of $CHCl_3$ was added 0.3

g of 85% *m*-chloroperbenzoic acid (14.8 mmol). The mixture was stirred at room temperature for 18 h. An additional 50 mg of 85% *m*-chloroperbenzoic acid was added and the stirring was continued for 1 h. The solid was collected by filtration and was washed with $CHCl_3$. This procedure gave 0.386 g (74%) of **26** as a white powder: mp 245–247 °C; NMR (Me_2SO-d_6) δ 4.64 (d, 1 H, J = 14 Hz, CH_AH_BSO), 5.07 (d, 1 H, J = 14 Hz, CH_AH_BSO), 7.4–7.7 (m, 3 H, 7-, 8-, 9-H), 7.72 (s, 1 H, N=CH), 7.9 (dd, 1 H, J_o = 9 Hz, J_m = 2 Hz, 2-H), 8 (m, 1 H, 10-H), 8.12 (d, 1 H, J_m = 2 Hz, 4-H), 8.21 (d, 1 H, J_o = 9 Hz, 1-H), 12.5 (br s, 1 H, NH); *m/e* 353 (M^+). Anal. ($C_{17}H_{11}N_3O_4S$) C, H, N.

R_m Value Determination. The general procedure of Biagi et al.[19] was used, except that 7% (v/v) Dow Corning 200 (50 cSt) in hexane was used to coat the Analtech silica gel GF plates and 15, 20, 25, or 30% (v/v) CH_3CN in pH 7.0 (0.01 M) phosphate buffer was used as the mobile phase. R_m vs. percent (v/v) of CH_3CN curves were parallel for all compounds, but values in 25% (v/v) CH_3CN are reported because the 0% (v/v) extrapolated values would be too high for these lipophilic compounds and consequently much less accurate than values at a fixed percent (v/v) of CH_3CN.

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Piperazinylpyrazines with Central Serotoninmimetic Activity

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A series of 2-(1-piperazinyl)pyrazines was synthesized and evaluated for central serotonin-like activity. The most interesting member of the series, 6-chloro-2-(1-piperazinyl)pyrazine (**3a**), had pharmacological properties characteristic of potent central serotoninmimetic activity and only weak peripheral serotoninmimetic action. Structural similarities between **3a** and serotonin are discussed.

Evidence that serotonin plays an important role in the physiology of the normal mammalian central nervous system as well as in certain pathological states is increasing steadily.[1,2] A number of compounds of diverse structure have been reported recently[3-10] to enhance central serotonin function by selectively inhibiting reuptake of serotonin or by acting as serotonin agonists. Prior to these reports we had begun a search for substances which enhance central nervous system serotonin function because of their potential utility in the treatment of depression, obesity, and sleep disorders. Our approach utilized the mouse head twitch assay[11] as a measure of central serotoninmimetic activity and contraction of the isolated rat uterus[12] as a measure of unwanted peripheral serotoninmimetic activity.

Several other classes of aryl- and heteroarylpiperazines with central serotoninmimetic activity have been discovered during the course of this work, the most promising of which were the piperazinylpyrazines. One of these, 6-chloro-2-(1-piperazinyl)pyrazine (**3a**), notable for its selectivity for the central nervous system, was selected as a clinical candidate.

In this paper we describe the synthesis of selected piperazinylpyrazines and give evidence for their selective central serotoninmimetic action compared with quipazine.[3] Further details of potential advantages over other serotonin-like compounds of current interest (e.g., fenfluramine and quipazine) will be published elsewhere.

Chemistry. Most of the piperazinylpyrazines of Table I were synthesized readily by reaction of the appropriate chloropyrazine **1** with piperazine or an N-substituted derivative **2**. Formation of bis(pyrazinyl)piperazines **4** proved to be a troublesome side reaction when piperazine itself was used, since the bis products were frequently difficult to separate completely from **3**. In some cases, e.g., **3d** and **3j** (Table I), remaining traces of the bis products were removed through chromatography over neutral Al_2O_3. Formation of **4** was found to be repressed, but not eliminated, through use of excess piperazine or by conducting the reaction *neat* at the melting point of piperazine.

Intermediate chloropyrazines **1** required for synthesis of **3b,g,h,j,o,p** were prepared from the corresponding 2-pyrazinones **5** by treatment with $POCl_3$ or, in the case

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