showed an improvement in the antitriglyceridemic activity with a loss of anticholesterolemic activity (compare **19** to **14**). The propionic acid derivative of glutarimide (**20**) showed significantly less antihypolipidemic activity than the corresponding 1,8-naphthalimide derivative (**15**). The butanone derivative of glutarimide (**21**) demonstrated decidedly less antitriglyceridemic activity compared to **16**; nevertheless, **21** showed slightly improved anticholesterolemic activity compared to **16**.

Whereas none of the new derivatives were as potent as the parent compound, phthalimide (**1**), in hypolipidemic activity, several agents demonstrated potent activity. The pentyl derivative of succinimide (**8**) demonstrated the most consistent activity and was impressive compared to clofibrate. This analogue warrants further study as a possible hypolipidemic agent.

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In Vitro Antimicrobial Activity of Benzoquinolinediones

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Abstract: The *in vitro* antibacterial and antifungal activity of benz[*g*]isoquinoline-5,10-dione (**1**), benzo[*g*]quinoline-5, 10-dione (**2**), benzo[*g*]quinoline-5,6-dione (**3**), and anthraquinone (**4**) was determined using the agar well-diffusion assay. The minimum inhibitory concentrations (MIC's) of each of the active compounds (**1–3**) was determined using the two-fold serial dilution technique. Of the four compounds tested, benz[*g*]isoquinoline-5,10-dione exhibited the best overall activity against both bacteria and fungi. Particularly noteworthy was its significant antifungal activity which was comparable to the activity of the standard antifungal antibiotic amphotericin B.

Walton (1) first reported in 1981 that commercial reagent grade samples of acridine were teratogenic to the cricket, *Acheta domesticus* (L.), and that the teratogenicity was due to impurities in the samples. The substance primarily responsible for the teratogenicity of commercial reagent grade acridine was later isolated and identified as benz[*g*]isoquinoline-5, 10-dione (**1**), a benzoquinolinedione isomer, which was estimated to be present at a concentration of only 20 ppm (2). In another study (3), compound **1** was shown to have potent teratogenic and embryotoxic activity in *A. domesticus*. At a dose of 0.1 ng/egg, compound **1** was capable of producing morphological abnormalities in cricket embryos. Furthermore, of three structurally related compounds (**2, 3** and **4**) tested for their teratogenicity, only benz[*h*]quinoline-5,6-dione (**3**) has been shown to be moderately active.

Elsewhere, it has been reported (4) that compound **3** showed relatively good *in vitro* activity against Ehrlich ascites tumor cells and Sarcoma 180 ascites tumor cells. It was also reported (5) that compound **3** was moderately active against certain strains of *Staphylococcus, Shigella* and *Salmonella.*

As one part of a study to ascertain the complete spectrum of biological activity of compound I and related benzoquinolinediones, the antimicrobial activities of each of the compounds was determined.

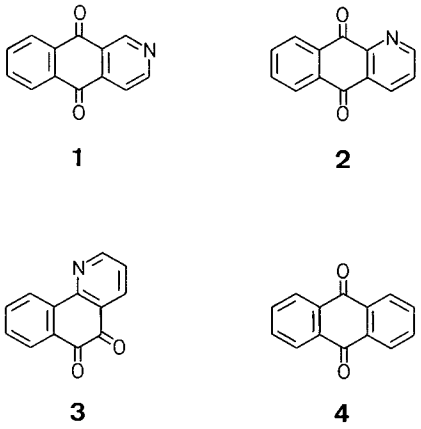

Fig. 1 Structures of benz[*g*]isoquinoline-5, 10-dione, benzoquinolinediones and anthraquinone.

Materials and Methods

Source of compounds: Benz[*g*]isoquinoline-5,10-dione (**1**) and benzo[*g*]-quinoline-5,10-dione (**2**) were synthesized using the method of Philips (6). The synthesis of benzo[*h*]quinoline-5, 10-dione (**3**) was carried out according to Skraup and Cobenzl (7). Anthraquinone (**4**) was obtained from Aldrich Chemical Company (Milwaukee, Wisconsin). All compounds were purified by preparative high pressure liquid chromatography to 99% or more, and

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their identities were confirmed by mass spectrometry, infrared and ^{1}H, ^{13}C-nuclear magnetic resonance spectra.

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), *Aspergillus niger* (ATCC 16888), *Trichophyton mentagrophytes* (ATCC 9972), *Helminthosporium* sp. (ATCC 4671), and *Polyporus sanguineus* (ATCC 14622). Routine qualitative screening of compounds was accomplished by an agar well-diffusion assay as previously described (8). All compounds were tested at a concentration of 1 mg/ml. Antimicrobial activity was recorded as the width (in mm) of the inhibition zone measured from the edge of the agar well to the edge of the inhibition zone.

Quantitative antimicrobial assay: For compounds that showed significant activity in the qualitative screen, the MIC values were determined using the two-fold serial dilution technique as previously described (8). All compounds were tested using 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 *S. aureus* and *P. aeruginosa* were incubated at 37°C for 24 h, while *M. smegmatis* cultures were incubated at 37°C for 48 h. Tubes inoculated with *B. subtilis* were incubated at 30°C for 24 h, while tubes inoculated with fungi and yeasts were incubated at 30°C for 48 h. Streptomycin and amphotericin B were used as standard antibiotics for comparison with the quinones.

Results and Discussion

All four compounds were tested qualitatively for antimicrobial activity against gram-positive and gram-negative bacteria, an acid-fast bacterium, yeasts and filamentous fungi using the agar well diffusion assay. Compound **1** and benzoquinolinediones **2** and **3** exhibited significant antibacterial (Table I) and antifungal (Table II) activity while anthraquinone (**4**) showed no significant activity against any of the test organisms. Streptomycin sulfate and amphotericin B were included in the assay to serve as antibacterial and antifungal standards, respectively. Compound **1** and the quinones **2** and **3** each had activity comparable to streptomycin sulfate against the gram-positive bacterium, *Staphylococcus aureus*, and the acid-fast bacterium *Mycobacterium smegmatis* (Table I). Compounds **1** and **2** exhibited activity comparable to the standard against the gram-positive *Bacillus subtilis* (Table I).

Perhaps more interesting than the antibacterial activity is the significant antifungal activity exhibited by compounds **1–3** in the agar well diffusion

Table I. Antibacterial Activity of Compounds **1–4**

Compound	Zone of inhibition[a]				
	B. subtilis	*S.aureus*	*E. coli*	*P. aeruginosa*	*M. smegmatis*
1	14	12	2	4	25
2	7	9	–	–	10
3	5	14	–	–	12
4	2	2	–	–	–
Streptomycin sulfate	10	8	8	7	18

[a]Zones are measured in mm from the edge of the well to the edge of the zone.

Table II. Antifungal Activity of Compounds **1–4**

Compound	Zone of inhibition[a]					
	C. albicans	*S. cerevisiae*	*A. niger*	*T. mentagrophytes*	*Helmintho sporium sp.*	*P. sanguineus*
1	11	16	7	12	20	16
2	7	8	3	6	16	12
3	7	7	7	20	22	16
4	5	5	–	–	–	7
Amphotericin B	10	14	9	16	15	14

[a]Zones are measured in mm from the edge of the well to the edge of the zone.

Table III. Minimum Inhibitory Concentrations[a] of Compounds **1–3** Against Bacteria

Compound	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*M. smegmatis*
1	1.56 (3.12)	3.12 (3.12)	25	1.56 (0.78)
2	3.12 (6.25)	6.25 (6.25)	NT[b]	12.5 (12.5)
3	NT[b]	1.56 (3.12)	NT[b]	25
Streptomycin sulfate	1.56	12.5	25	0.78

[a]MIC's are expressed as μg/ml. Numbers in parentheses refer to values obtained on duplicate testing.
[b]NT = not tested

Table. IV. Minimum Inhibitory Concentrations[a] of Compounds **1–3** Against Fungi

Compound	*C. albicans*	*S. cerevisiae*	*A. niger*	*T. mentagrophytes*	*Helmintho-sporium sp.*	*P. sanguineus*
1	6.25 (6.25)	3.12 (3.12)	12.5 (12.5)	1.56 (1.56)	1.56 (1.56)	3.12 (3.12)
2	25	6.25 (6.25)	NT[b]	NT[b]	1.56 (1.56)	25
3	NT[b]	25	50	12.5 (12.5)	6.25 (3.12)	25
Amphotericin B	6.25	1.56	12.5	6.25	3.12	1.56

[a]MIC's are expressed as μg/ml. Numbers in parentheses refer to values obtained on duplicate testing.
[b]NT = not tested

assay (Table II). Compound **1** seemed especially active against the broad range of fungal test organisms. Benzoquinolinediones **2** and **3** also exhibited some antifungal activity, especially against the filamentous fungi *Trichophyton mentagrophytes, Helminthosporium* sp., and *Polyporus sanguineus* (Table II).

The results of the qualitative agar well-diffusion assay prompted a study to determine the minimum inhibitory concentrations (MICs) of each of the active compounds for each susceptible organism. The results of the MIC determinations correlate well with the initial qualitative results. Compound **1** had activity comparable to streptomycin sulfate against all of the bacterial test organisms (Table III). The MIC values for **2** and **3** were comparable to streptomycin sulfate for *B. subtilis* and *S. aureus*, but both **2** and **3** appear to be less active against *M. smegmatis* (Table III).

Based on a comparison of MIC values, compound **1** is at least comparable in antifungal activity to the standard amphotericin B against all of the fungal test organisms (Table IV). The benzoquinolinediones **2** and **3**, however, appear to be slightly less effective than either amphotericin B or compound **1** against most of the fungal test organisms (Table IV).

Benz[*g*]isoquinoline-5,10-dione (**1**) appears to have the most overall activity of the compounds tested for antibacterial and antifungal activity. Benzo[*g*]-quinoline-5,10-dione (**2**), which differs from **1** only in the position of the heterocyclic nitrogen, has a narrower spectrum of activity than **1**, especially as an antifungal agent, but is comparable in its quantitative activity, whereas benzo[*h*]quinoline-5,6-dione (**3**) is less active than either **1** or **2**. Anthraquinone (**4**), which does not possess a nitrogen-containing heterocyclic ring, was essentially inactive. Clearly, the presence of the nitrogen heterocyclic ring is important for antimicrobial activity and both the spectrum and degree of activity appear to be related to the position of the nitrogen in the heterocyclic ring. The antimicrobial activity appears also to be affected by the ring structure and/or quinone system present since **3** was less active than either **1** or **2**.

The antimicrobial activities correlate well with other studies on the biological activities of benzo[*g*]isoquinoline-5,10-dione (**1**) and its benzoquinolinedione isomers (**2, 3**) in which it was found that **1** was the most teratogenic and embryotoxic of the three compounds studied (3). Although the benzoquinolinediones have exhibited some interesting biological activities, there have been as yet no toxicological studies on these compounds.

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Phosphorus GABA Analogues as Potential Prodrugs

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Abstract: Analogues of γ-aminobutyric acid (GABA), wherein a P=O moiety is separated by three carbon atoms from an amino group, were incorporated into Schiff bases as potential acid-labile carrier molecules. These include 3-aminophenylphosphonic acid, its dimethyl ester and its previously unreported *N,N'*-diisopropylphosphonodiamide. A benzophenone derivative of GABA was also synthesized. A study of the degrees of *in vitro* hydrolysis of four Schiff bases indicated that lability of the C=N bond is determined by eletronic influences of ring substituents. All new products were tested for abilities to inhibit maximal electroshock- and subcutaneous pentylenetetrazol (Metrazol)-induced seizures in mice. Activity was found only in the former system with moderate inhibition displayed by two dimethyl ester and the GABA Schiff bases.

Anticonvulsant activity has recently been found to be associated with esters (**1b–1d**) of 3-aminophenylphosphonic acid **1a** (1, 2). In this report we describe the synthesis and testing of an additional derivative **1e** and Schiff bases (anils) **2a–e**, the latter being prepared by the incorporation of **1d** and **1a** into benzylidene derivatives. The 2,2'-dihydroxybenzophenone anil of γ-aminobutyric acid (GABA) **3** was also included in this study. All new products were tested for abilities to inhibit seizures induced in mice by maximal electroshock (MES) and subcutaneously administered pentylenetetrazol (Metrazol) (scMet) and for neurotoxicity. In addition, the degree of *in vitro* hydrolysis of four derivatives was investigated.

Background

The concept of Schiff bases as carrier molecules for biologically active amines has been applied in several previous investigations. Examples of these are cancer chemotherapeutic agents (3–5) and, more recently, in central nervous system (CNS) depressants (6) and GABA and its amide (GABAMIDE) (7, 8). GABA acts as an inhibitory neurotransmitter in the CNS and an

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