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Synthesis of *as*-Triazines as Potential Antiviral Agents

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Abstract □ Four acenaphtho[1,2-*e*]-*as*-triazines and 11 5,6-diaryl-*as*-triazines, all substituted with an aliphatic or aromatic amino function in the 3-position, were synthesized. Two acenaphthotriazines were active against vesicular stomatitis virus in tissue culture.

Keyphrases □ *as*-Triazines, various substituted—synthesized, antiviral activity evaluated □ Antiviral activity—various substituted *as*-triazines evaluated □ Structure–activity relationships—various substituted *as*-triazines evaluated for antiviral activity

The broad spectrum antiviral activity of the 5*H*-*as*-triazino[5,6-*b*]indoles (1–3), as well as reports of antiviral activity of other *as*-triazines (4–6), prompted preparation of a series of related analogs for evaluation as potential antiviral agents. This effort consisted of the synthesis of two series, one containing a coplanar ring system and the other containing a similar number of aromatic rings but not in a coplanar arrangement.

The first series, 9-substituted acenaphtho[1,2-*e*]-*as*-triazines (Table I), is modeled after the 5-alkyl-*as*-triazino[5,6-*b*]indoles in that a hydrophobic moiety (the acenaphtho ring) is substituted for the alkylindolo ring. The resulting compounds contain a planar aromatic surface, similar to that of the *as*-triazinoindoles, but lacking the steric factors contributed by the alkyl group, which can rotate above and below the plane of the fused rings.The second series of compounds, 3-substituted 5,6-diaryl-*as*-triazines (Table II), was prepared to determine the necessity of the planar fused rings in antiviral activity by building a system that could not attain a coplanar configuration. All compounds (Tables I and II) were substituted with alkyl or aryl amino side chains, which have been demonstrated to be efficacious in antiviral triazines (2, 3).

RESULTS AND DISCUSSION

The synthetic pathways utilized in the preparation of I–XX are similar and consist of the acid- or base-catalyzed condensation of either thiosemicarbazide or semicarbazide hydrochloride with acenaphthoquinone or the appropriately substituted benzil. Products of the thiosemicarbazide–quinone condensation could be treated directly with the desired alkyl or aryl amine to produce the corresponding derivatives, II–V. However, the 5,6-diaryl-*as*-triazin-3(2*H*)-ones do not react with the amines, necessitating their conversion to the corresponding 3-chloro derivatives by reaction with phosphoryl chloride. The enhanced reactivity of 3-chloro-5,6-diaryl-*as*-triazines allowed their facile conversion to X–XX.

Compounds III–V, X–XV, and XVII–XX were subjected to both *in vitro* and *in vivo* antiviral activity screens as described under *Experimental.* A tissue culture evaluation of III and IV indicated activity when the compounds were exposed to cells challenged with vesicular stomatitis virus, a single-stranded RNA rabies-like virus, as determined by the dye retention assay. The activity of IV against vesicular stomatitis virus (active at 25 μg/ml of culture medium) was greater than that of III (active at 50 μg/ml); however, IV also exhibited a greater toxicity (50 μg/ml) to cell cultures than did III (toxic at 250 μg/ml). Other compounds failed to demonstrate any significant *in vitro* activity against vesicular stomatitis virus.

All compounds were judged inactive when tested *in vitro* (under similar conditions) against the following viruses: respiratory syncytial, parainfluenza-3, herpes simplex, rhinovirus-14, shipping fever (bovine parainfluenza-3), and Newcastle disease.

The *in vivo* antiviral evaluation system consisted of an encephalomyocarditis virus-in-mouse screen in which activity is determined by a prolongation of survival time in the treated animals. None of the compounds demonstrated activity.

Because several related 3,5-diamino-*as*-triazines (7) have exhibited antimalarial activity, X–XX also were evaluated for *in vivo* antimalarial activity in mice infected with *Plasmodium berghei* by the method of Osdene *et al.* (8). None of the compounds showed any significant activity in the malaria screen.

EXPERIMENTAL[1]

All melting points were obtained using an oil bath melting-point apparatus and are uncorrected. Satisfactory IR spectra were obtained, and the expected PMR spectra were obtained in deuterochloroform (tetramethylsilane standard).

Acenaphtho[1,2-*e*]-*as*-triazine-9(8*H*)-thione (I)—Attempts to prepare this compound by the method reported for the corresponding

[1] Elemental analyses were determined by Atlantic Microlab, Atlanta, Ga.

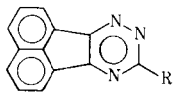


Table I—9-(3-Substituted Propylamino)acenaphtho[1,2-*e*]-*as*-triazines[a]

Number	R	Melting Point	Yield, %	Formula	Analysis, % Calc.	Found
II	$NH(CH_2)_3OH$	201–202°	46	$C_{16}H_{14}N_4O$	C 69.16	69.16
					H 5.04	5.25
III	$NH(CH_2)_3N(CH_3)_2$	173–174.5°	68	$C_{18}H_{19}N_5$	C 70.82	70.71
					H 6.23	6.39
IV	$NH(CH_2)_3N(C_2H_5)_2$	115–116°	62	$C_{20}H_{23}N_5$	C 72.07	71.91
					H 6.91	7.01
V	$NH(CH_2)_3N(C_2H_5)_2$	92–93°	56	$C_{24}H_{31}N_5$	C 74.07	74.14
					H 7.97	7.93

[a] All compounds were recrystallized from acetonitrile.

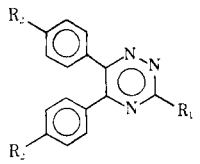
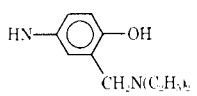
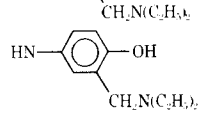


Table II—3-Substituted 5,6-Diaryl-*as*-triazines

Number	R_1	R_2	Melting Point	Yield, %	Formula	Analysis, % Calc.	Found
X[a]	$NH(CH_2)_3OH$	H	137–138°	92	$C_{18}H_{18}N_4O$	C 70.59	70.30
						H 5.88	6.09
						N 18.30	18.07
XI[a]	$NH(CH_2)_3N(C_2H_5)_2$	H	112–114°	64	$C_{22}H_{27}N_5$	C 73.13	73.08
						H 7.49	7.56
						N 19.39	19.23
XII[a]	$NH(CH_2)_3N(C_4H_9)_2$	H	69–70.5°	69	$C_{26}H_{35}N_5$	C 74.82	74.89
						H 8.39	8.42
						N 16.79	16.79
XIII[a]	$NH(CH_2)_3OH$	Cl	169–170°	87	$C_{18}H_{16}Cl_2N_4O$	C 57.60	57.46
						H 4.27	4.20
XIV[b]	$NH(CH_2)_3N(C_2H_5)_2$	Cl	127–128°	56	$C_{22}H_{25}Cl_2N_5$	C 61.40	61.30
						H 5.81	6.05
XV[b]	$NH(CH_2)_3N(C_4H_9)_2$	Cl	104–105.5°	62	$C_{26}H_{33}Cl_2N_5$	C 64.20	64.21
						H 6.79	6.85
XVI[b]	$NHCHCH_3$ | $(CH_2)_3N(C_2H_5)_2$	Cl	87–90°	56	$C_{24}H_{29}Cl_2N_5$	C 62.88	62.94
						H 6.33	6.48
						N 15.28	15.23
XVII[a]	3-Hydroxyanilino	H	253–254.5°	65	$C_{21}H_{16}N_4O$	C 74.12	74.16
						H 4.71	4.76
						N 16.47	16.53
XVIII[b]	3-Hydroxyanilino	Cl	274–275.5°	88	$C_{21}H_{14}Cl_2N_4O$	C 61.60	61.87
						H 3.42	3.34
XIX[c]		H	198–200°	52	$C_{26}H_{27}N_5O$	C 73.41	73.54
						H 6.35	6.37
						N 16.47	16.51
XX[b]		Cl	230–232°	22	$C_{26}H_{25}Cl_2N_5O$	C 63.16	63.22
						H 5.06	5.19

[a] Recrystallized from ethanol. [b] Recrystallized from acetonitrile. [c] Recrystallized from benzene.

phenanthro[5,6-*e*]-*as*-triazine (9) resulted in poor yields, but the procedure outlined by Gladych *et al.* (2) for the synthesis of the analogous indolo[2,3-*e*]-*as*-triazine afforded good yields. Thus, a mixture of thiosemicarbazide (1 g, 0.011 mole), acenaphthoquinone (1.7 g, 0.093 mole), and sodium carbonate (2.2 g, 0.02 mole) was refluxed for 9 hr in 50 ml of water, filtered hot, cooled, and then acidified with acetic acid. The precipitate was collected, recrystallized once from acetonitrile (85% yield, mp 272–273°), and used in subsequent synthesis without further purification. The analytical sample melted at 275–276°.

Anal.—Calc. for $C_{13}H_7N_3S$: C, 65.82; H, 2.95; N, 17.72. Found: C, 65.74; H, 2.95; N, 17.68.

9-(3-Substituted Propylamino)acenaphtho[1,2-*e*]-*as*-triazines (II–V, Table I)—In a typical reaction, a mixture of I (2 g, 0.0084 mole) in 10 ml of *N',N'*-diethylaminopropane-1,3-diamine was refluxed for 5 hr or until evolution of hydrogen sulfide gas had ceased (detected with lead acetate test paper). After cooling, the mixture was poured into 100 ml of cold water. The precipitate was collected and recrystallized from acetonitrile to yield 65% 9-(3-diethylaminopropylamino)acenaphtho[1,2-*e*]-*as*-triazine (IV).

5,6-Diaryl-*as*-triazin-3(2*H*)-ones (VI and VII)—Utilizing the method described by Blitz (10), these compounds were obtained by refluxing a mixture of the appropriate benzil [prepared by the benzoin condensation (11) followed by oxidation (12)] with an equimolar amount of semicarbazide hydrochloride in acetic acid for 3 hr, with subsequent precipitation of the product by addition of water. Recrystallization from ethanol gave a melting point of 223–225° (59% yield) for the 5,6-diphenyl derivative (VI) [lit. (11) mp 224–225°].

3-Chloro-5,6-diaryl-*as*-triazines (VIII and IX)—In a modification (13) of the reported procedure (9), the 3-chloro analogs of VI and VII were prepared by slowly adding phosphoryl chloride (5 ml) to the appropriate 3(2*H*)-one derivative (0.0031 mole) in 1–2 ml of *N,N*-dimethylaniline and refluxing the resulting combination for 1 hr or until hydrogen chloride gas evolution had ceased. After cooling, the mixture was cautiously poured onto 500 g of ice with vigorous stirring. The resulting precipitate was collected and triturated with 1 *M* NaOH before being recrystallized from methanol to yield 51% of VIII, mp 156–157° [lit. (9) mp 157°]. Similarly, starting with VII (mp 238–240°), which was used directly without further characterization, a yield of 30% of the 5,6-bis(4-chloro-

phenyl) derivative (IX), mp 165–167°, was obtained.

Anal.—Calc. for $C_{15}H_8Cl_3N_3$: C, 53.49; H, 2.38. Found: C, 53.57; H, 2.47.

3-(3-Substituted Alkylamino)-5,6-diaryl-*as*-triazines (X–XV, Table II)—The method for preparing these compounds was essentially identical to that employed for the analogous acenaphtho[1,2-*e*]-*as*-triazines II–V, except that reaction times were decreased to 30 min because of an enhanced ease of displacement of the 3-chloro moiety.

3-(5-Diethylamino-2-pentylamino)-5,6-bis(4-chlorophenyl)-*as*-triazine (XVI, Table II)—This compound was prepared in a manner analogous to that described for X–XV, except that the solvent was removed *in vacuo* after reflux and the resulting oil was triturated with dilute base (1 *M* NaOH), filtered, and recrystallized from acetonitrile, mp 87–90° (56% yield).

3-Substituted Arylamino-5,6-diaryl-*as*-triazines (XVII–XX, Table II)—The general procedure for the preparation of 3-arylamino analogs of VIII and IX was as follows. To a mixture of VIII in toluene (0.0075 mole in 10 ml) was added an excess of 3-hydroxyaniline, and the resulting slurry was refluxed for 3–6 hr and poured into cold water. The resulting precipitate was collected. Recrystallization from ethanol or acetonitrile afforded a 65% yield of XVII, mp 253–254.5°. The synthesis of 3-(α-diethylaminomethyl)-4-hydroxyaniline was conducted as described by Burckhalter *et al.* (14); the product, without isolation, was allowed to react directly with the desired triazine (VIII or IX) in aqueous solution to yield XIX (52% yield) and XX (22% yield).

Antiviral Evaluation Screens—*Method A: Plaque Reduction Assay*—This system was utilized in the antiviral evaluation of compounds *versus* herpes simplex, parainfluenza-3, and respiratory syncytial viruses. Compounds were suspended in a culture medium and applied to a representative population in a series of replicate human epidermoid larynx carcinoma cell cultures known to be susceptible to the challenge viruses. An appropriate number of cultures were maintained as inoculated, but untreated, controls; others were exposed to the compound alone (to ascertain toxicity); still others remained as untreated and uninfected control cultures.

All cell cultures were incubated at 35° for the duration of the experiments. Upon termination of the experiment, the cultures were stained with neutral red (a vital stain) and examined for compound toxicity. Cultures that, by visual inspection, stained less intensely than the untreated controls were considered to have received a toxic dose of the compound in question and were subsequently disregarded. The cell cultures were then fixed in formalin and stained with gentian violet, and the number of viral-produced plaques was determined. An active compound was defined as one in which at least two nontoxic concentrations resulted in a 50% or greater plaque reduction in treated cell cultures.

Method B: Dye Retention Assay—Viruses evaluated with this system were rhinovirus-14 (propagated in human epitheloid cervix carcinoma cells), shipping fever and vesicular stomatitis (propagated in canine kidney cells), and Newcastle disease (propagated in mouse connective tissue). This evaluation procedure was essentially the same as Method A, except that, instead of subsequent staining with gentian violet, the cultures were washed with buffered saline after being stained with neutral red and the retained dye was then extracted with buffered methanol–formalin.

By this procedure, the amount of retained dye can be quantitatively determined by spectrophotometric methods. Active, nontoxic compounds were those in which the treated cell cultures retained at least 75% of the dye (neutral red), as judged on a scale where uninfected controls demonstrated 100% dye retention and virus-infected controls showed 0% dye retention.

Method C: Encephalomyocarditis Virus/Mouse Assay—In this system, mice were injected subcutaneously with a controlled titer of encephalomyocarditis virus adjusted to produce death after 4–5 days in untreated control animals. Compounds to be screened were suspended in a 0.4% 2-methoxyethanol[2]–0.5% polysorbate 80[3] solution at 10 mg/ml. Test animals were inoculated either intraperitoneally or subcutaneously with this suspension 1 hr prior to virus administration. A compound was judged active when it produced a mean survival time of 12–14 days in treated animals. The mean survival time controls usually died at 4.5 days. The validity of the assay was demonstrated by production of a control group of mice pretreated with 10 μg of poly(I)·poly(C), an interferon inducer.

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[2] Methocellosolve.
[3] Tween 80.