Synthesis, Reactions and Biological Evaluation of Some 1,2,4-Triazine Derivatives

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6-Substituted benzyl-4-phenyl-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-5-ones **3a-d** were prepared and converted into their corresponding 3-methylthio derivatives **4a-d**. Reaction of compounds **4a-d** with hydrazine hydrate gave the corresponding 4-amino-3-anilino-4,5-dihydro-1,2,4-triazin-5-ones **5a-d**. 6-Substituted benzyl-4-phenyl-2,3,4,5-tetrahydro-1,2,4-triazin-3,5-diones **9a-c** were synthesized and allowed to react with hydrazine hydrate to give the corresponding 6-substituted benzyl-4-amino-2,3,4,5-tetrahydro-1,2,4-triazin-3,5-diones **10a-c**. The biological evaluation of some of these triazines is described. All compounds were screened for antiviral, antibacterial, antimycobacterial, antifungal and antiyeast activity. No important biological activity was found.

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Chemistry.

The synthesis of 1,2,4-triazines is of considerable importance, since some of these compounds have promising biological activity [1-7]. This encouraged us to continue our previous work in this field [6] [8]. We now have studied the condensation reaction between arylpyruvic acids and 4-phenylthiosemicarbazide and some reactions of the condensation products. Thus, when the arylpyruvic acids **2a-d** were allowed to react (without isolation, in the hydrolysis medium of the corresponding azlactones **1a-d**) with 4-phenylthiosemicarbazide at *p*H about 5, they yielded the corresponding 6-substituted benzyl-4-phenyl-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-5-ones **3a-d**. When compounds **3a-d**, in methanolic sodium methoxide solution, were treated with methyl iodide they afforded the 3-methylmercapto derivatives **4a-d**.

The action of hydrazine hydrate on the 3-methylmercaptotriazines **4a-d** led to the formation of the corresponding 4-amino-3-anilino-4,5-dihydro-1,2,4-triazin-5-ones **5a-d**. All attempts to isolate any intermediate in this reaction were unsuccessful. The structure of compounds **5a-d** was established by their independent synthesis from 4-amino-3-methylmercapto-4,5-dihydro-1,2,4-triazin-5-ones **6a-d**, by heating with aniline at 180°. Furthermore, the products **5a-d** were readily deaminated by the action of nitrous acid to give the corresponding 3-anilino-2,5-dihydro-1,2,4-triazin-5-ones **7a-d** which are identical with the same products obtained previously from aniline and compounds **8a-d** [8].

The synthesis of the new 6-substituted benzyl-4-phenyl-2,3,4,5-tetrahydro-1,2,4-triazin-3,5-diones **9a-c** has, now, been achieved either by hydrolysis of compounds **4b-d** or by direct condensation of the arylpyruvic acids **2b-d** and 4-phenylsemicarbazide. When the triazinediones **9a-c** were treated with hydrazine hydrate in refluxing ethanol, they yielded the corresponding 6-substituted benzyl-4-amino-2,3,4,5-tetrahydro-1,2,4-triazin-3,5-diones **10a-c**. The structure of compounds **10a-c** was confirmed by their independent synthesis either by hydrolysis of compounds **6b-d** or by condensing the appropriate arylpyruvic acids with carbohydrazide.

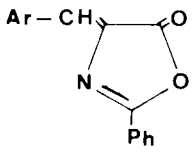
Microbiology.

All compounds were tested for antiviral, antibacterial, antimycobacterial, antiyeast and antifungal activity.

The "endpoint titration technique" in liquid medium (EPTT) [9] was employed to screen the prepared compounds for antiviral activity. The activity against Herpes simplex virus type 1 (code 1023) (10^6TCD_{50}/ml) and against polio type 1 (10^7TCD_{50}/ml) was determined.

Monolayers of VERO-cells were grown in microtiter plates. The cells were infected with 100 μl of tenfold dilutions of the original virus sample. The plates were incubated at 37° for 1 hour to allow virus absorption to the cells. Stock solutions of the compounds were prepared using DMSO and adding maintenance medium up to concentration of 200 μl/ml. After adsorption period defined compound dilutions were added.

Cytotoxicity, cell- and virus growth controls were studied in the same microtiter plate. The plates were incubated at 37° (humid circulation) for several days. The specific cytopathogenic effect caused by the viral infection was investigated by inversed light-microscopy. The antiviral activity is expressed as the virus titer reduction factor, *i.e.* the ratio of virus titer in the control to the virus titer in the presence of the highest non-cytotoxic concen-



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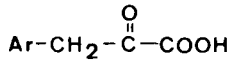

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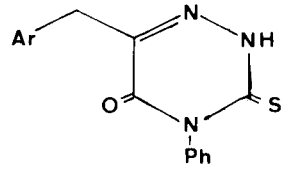

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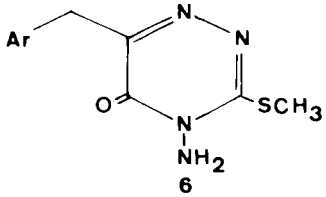

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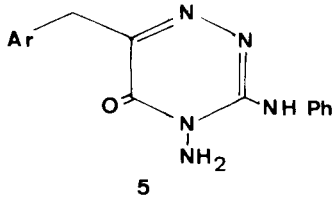

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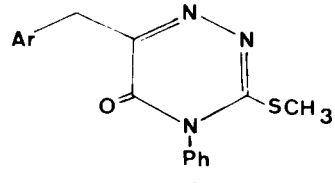

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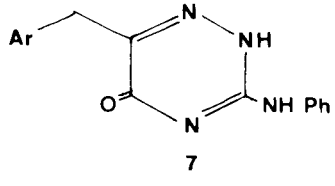

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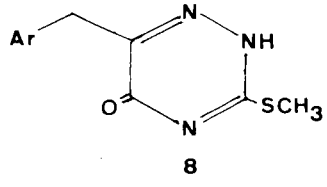
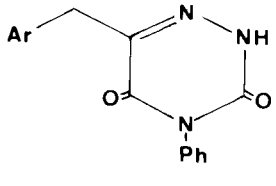


8

a, Ar = p-$CH_3OC_6H_4$
c, Ar = 3,4-$(CH_3O)_2C_6H_3$

b, Ar = p-Cl-C_6H_4
d, Ar = 3,4-$(OCH_2O)C_6H_3$



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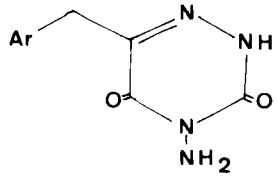

10

a, Ar = p-Cl-C_6H_4
b, Ar = 3,4-$(CH_3O)_2C_6H_3$
c, Ar = 3,4-$(OCH_2O)C_6H_3$

tration of compounds. A reduction factor of at least 10^3 is considered to be significant. All compounds however had a reduction factor equal to 1, which means no antiviral activity. Compounds **4** however showed a high cytotoxicity. The maximal not toxic dose was smaller than 1 μg/ml. These cytotoxic effects are now under further investigation.

The agar diffusion technique was employed to screen the triazines for antibacterial and antiyeast activity. The susceptibility of the following organisms was tested: *Staphylococcus aureus, Streptococcus viridans, Diplococcus pneumoniae, Enterococcus, Enterobacter cloacae, Enterobacter aerogenes, Klebsiella pneumoniae, Proteus vulgaris, Morganella morganii, Pseudomonas aeruginosa, Escherichia coli, Serratia marcescens, Salmonella* type B, *Candida albicans* and *Candida tropicalis.* All strains were maintained and tested on Diagnostica Sensitivity Test (DST) agar. *Streptococcus viridans* was grown and tested on DST agar supplemented with 5% horse blood. Inoculation was done with an inoculum consisting of approximately 10^6 cells/ml phosphate buffered saline solution. Two hundred μl of diluted DMSO solution of the compounds was put into cups, poored into the agar. After diffusion at 4° incubation was continued at 37° for 18 hours. The activity was evaluated by measuring the zone of inhibition around the cups. Neomycin and nystatin were used as references.

All triazines tested showed a neglectible activity against

Table

Compound	Mp °C	Yield %	Formula M.W.	Analysis % Calcd./Found C	H	N	S	Cl
3a	186	56	$C_{17}H_{15}N_3O_2S$	62.75	4.65	12.91	9.85	–
			(325.39)	63.00	4.50	13.30	9.90	–
3b	177	54	$C_{16}H_{12}N_3OSCl$	58.27	3.67	12.74	9.72	10.75
			(329.81)	58.60	3.61	12.90	10.00	11.10
3c	178	60	$C_{18}H_{17}N_3O_3S$	60.83	4.82	11.82	9.02	–
			(355.41)	60.60	4.70	11.80	8.70	–
3d	180	45	$C_{17}H_{13}N_3O_3S$	60.17	3.86	12.38	9.45	–
			(339.37)	60.40	3.70	12.70	9.20	–
4a	185	58	$C_{18}H_{17}N_3O_2S$	63.69	5.05	12.38	9.44	–
			(339.41)	64.00	5.20	12.70	9.30	–
4b	187	65	$C_{17}H_{14}N_3OSCl$	59.39	4.10	12.22	9.32	10.31
			(343.83)	58.90	4.50	12.50	8.90	10.11
4c	156	70	$C_{19}H_{19}N_3O_3S$	61.77	5.18	11.37	8.68	–
			(369.44)	62.20	5.30	11.60	8.40	–
4d	135	80	$C_{18}H_{15}N_3O_3S$	61.17	4.28	11.89	9.07	–
			(353.4)	61.30	4.15	11.50	9.20	–
5a [a]	194	(i) 61	$C_{17}H_{17}N_5O_2$	63.14	5.30	21.66	–	–
		(ii) 70	(323.36)	63.50	5.20	21.30	–	–
5b [a]	208	(i) 65	$C_{16}H_{14}N_5OCl$	58.63	4.30	21.36	–	–
		(ii) 71	(327.78)	58.60	4.10	21.50	–	–
5c [a]	167	(i) 62	$C_{18}H_{19}N_5O_3$	61.18	5.42	19.82	–	–
		(ii) 65	(353.38)	61.30	5.20	19.70	–	–
5d [a]	218	(i) 65	$C_{17}H_{15}N_5O_3$	60.53	4.48	20.76	–	–
		(ii) 70	(337.34)	60.40	4.50	21.10	–	–
9a [a]	240	(i) 46	$C_{16}H_{12}N_3O_2Cl$	61.25	3.85	13.39	–	11.30
		(ii) 79	(313.75)	61.50	3.40	13.56	–	11.40
9b [a]	250	(i) 35	$C_{18}H_{17}N_3O_4$	63.71	5.05	12.38	–	–
		(ii) 60	(339.35)	63.35	5.00	12.00	–	–
9c [a]	200	(i) 48	$C_{17}H_{13}N_3O_4$	63.15	4.05	13.0	–	–
		(ii) 80	(323.31)	62.86	3.90	13.1	–	–
10a [a]	193	(i) 79	$C_{10}H_9N_4O_2Cl$	47.54	3.59	22.17	–	14.03
		(ii) 81	(252.66)	47.20	3.40	21.80	–	14.26
10b [a]	176	(i) 75	$C_{12}H_{14}N_4O_4$	51.79	5.07	20.13	–	–
		(ii) 80	(278.27)	51.60	5.10	20.20	–	–
10c [a]	191	(i) 80	$C_{11}H_{10}N_4O_4$	50.38	3.84	21.37	–	–
		(ii) 82	(262.23)	50.80	4.10	21.70	–	–

[a] (i) and (ii), yield obtained by methods (i) and (ii) respectively.

the strains tested. Compounds **4** however showed an activity against *Streptococcus viridans*. This will be further investigated. The agar dilution technique was employed to screen the compounds for antifungal activity. The following fungi were used: *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Microsporum canis* and *Trichophyton mentagrophytes*. The cultures were maintained in slight acid nutrient broth at ambient temperature (permanently shaked). To elaborate the dilution technique sabouroud dextrose agar was distributed into test tubes and autoclaved. The compounds solution (*cf.* above) were thoroughly mixed at 45° with the agar. After solidifaction in declined position the mycelium (prepared by ultrasonication) was carefully spread on the agar surface and the tubes incubated at ambient temperature for 2 weeks.

The fungal growth was compared with the fungal growth in control tubes. Inhibition, reduced growth, retardation and normal growth were distinguished.

None of the triazine tested showed antifungal activity. The agar dilution method was also used for the test for antimycobacterial activity. The following mycobacteria were used: *M. phlei, M. chelonae, M. fortuitum, M. aureus, M. vaccae* (fast growing) and *M. mais, M. avium, M. intra-*

cellulare, M. thermoresistibile, M. tuberculosis (slow growing). Middlebrook 7H10 agar with 10% oleic acid, albumine, dextrose (OAD) solution was used for the fast growing mycobacteria; Middlebrook 7H9 agar with 10% OAD solution for the slow growing mycobacteria. Incubation time varied from 72 hours for the former to 1 week for the latter. The triazines tested showed only a slight activity against mycobacteria.

EXPERIMENTAL

All melting points are uncorrected. The ir spectra (potassium bromide pellets) were recorded on a Unicam SP 1200 spectrophotometer, or a Beckman Acculab-4 spectrophotometer. The ν max are given in cm^{-1}. The ^{1}H-nmr spectra were recorded on a Varian EM 360 A spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane.

6-Substituted Benzyl-4-phenyl-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-5-ones **3a-d**.

Each of the azlactones **1a-d** (0.1 mole) was heated under reflux in potassium hydroxide solution (0.3 mole, 1 *M* aqueous solution) for 6 hours. The reaction mixture was cooled, acidified with acetic acid, then phenylthiosemicarbazide (0.1 mole) in ethanol (300 ml) was added. The reaction mixture was boiled under reflux for 8 hours and left, at room temperature, overnight. The precipitate obtained was collected and crystallized from ethanol into colorless crystals of **3a-d** (*cf.* Table).

Compound **3c**.

This compound had ir: ν max 3250, 1690, 1490, 1260, 1235, 1025; ^{1}H-nmr (DMSO-d_6): δ 3.8 (s, 6H, CH_3), 3.9 (s, 2H, CH_2), 6.9 (m, 3H, arom), 7.4 (m, 5H, arom), 13.8 (s, 1H, NH).

6-Substituted Benzyl-4-phenyl-3-methylthio-4,5-dihydro-1,2,4-triazin-5-ones **4a-d**.

Each of compounds **3a-d** (0.01 mole) was dissolved in cold methanolic sodium methoxide solution (prepared from 0.23 g sodium metal and 10 ml absolute methanol), then methyl iodide (1.47 g) was added. The reaction mixture was shaken for 15 minutes and left overnight, at room temperature. The precipitate obtained was collected and recrystallized from ethanol into colorless crystals of **4a-d** (*cf.* Table).

Compound **4c**.

This compound had ir: ν max 1690, 1510, 1470, 1255, 1235; ^{1}H-nmr (DMSO-d_6): δ 2.5 (s, 3H, S-CH_3), 3.7 (s, 6H, CH_3-O), 4.0 (s, 2H, CH_2), 6.9 (m, 3H, O-arom), 7.6 (m, 5H, arom).

4-Amino-3-anilino-6-substituted benzyl-4,5-dihydro-1,2,4-triazin-5-ones **5a-d**.

Method (i).

A mixture of each of compounds **4a-d** (1.0 g) and hydrazine hydrate (2 ml, 99%) in isopropyl alcohol (10 ml) was boiled under reflux for 3 hours. The precipitate formed was collected, after cooling, and recrystallized from *N,N*-dimethylformamide into colorless crystals of **5a-d** (*cf.* Table).

Compound **5d**.

This compound had ir: ν max 3315, 3290, 1595, 1540, 1485, 1250, 755; ^{1}H-nmr (DMSO-d_6): δ 3.9 (s, 2H, CH_2-arom), 5.8 (s, 2H, NH_2), 6.0 (s, 2H, O-CH_2-O), 6.8 (m, 3H, arom), 7.4 (m, 2H, O-arom), 7.9 (m, 3H, O-arom), 9.2 (s, 1H, NH).

Method (ii).

A mixture of each compounds **6a-d** [8] (0.5 g) and aniline (2 ml) was heated under reflux for 15-30 minutes. It was then cooled and diluted with ethanol. The precipitate obtained was collected and recrystallized from *N-N*-dimethylformamide as colorless crystals of **5a-d**.

Each of compounds **5a-d** obtained by method (ii) showed identical melting point to the corresponding compound obtained by method (i). Melting points of a mixture of identical compounds obtained by both methods showed no depression. They showed identical ir spectra.

Deamination of Compound **5c**.

To a cold suspension of compound **5c** (0.2 g) in ethanol (10 ml) and concentrated hydrochloric acid (2 ml) was added cold sodium nitrite solution (5 ml, 10%) with shaking. The reaction mixture was allowed to stand overnight at room temperature. The precipitate formed, was collected and recrystallized from *N,N*-dimethylformamide into colorless crystals of compound **7c**, mp 232° (mixed mp with an authentic sample prepared from the reaction of aniline and compound **8c** showed no depression [8]).

6-Substituted Benzyl-4-phenyl-2,3,4,5-tetrahydro-1,2,4-triazin-3,5-diones **9a-c**.

Method (i).

From Arylpyruvic Acids **2b-d** and 4-Phenylsemicarbazide.

Each of the azlactones **1b-d** (0.1 mole) was hydrolysed as described before. After acidification with acetic acid, 4-phenylsemicarbazide (15.1 g in 300 ml of ethanol) was added. The reaction mixture was heated under reflux for 8 hours and allowed to stand at room temperature overnight. The precipitate obtained was collected and recrystallized from acetic acid into colorless crystals of **9a-c** (*cf.* Table).

Compound **9c**.

This compound had ir: ν max 3200, 3100, 2900, 1665, 1485, 1435, 1245, 1200; ^{1}H-nmr (DMSO-d_6): δ 3.8 (s, 2H, CH_2-arom), 6.1 (s, 2H, O-CH_2-O), 6.8 (m, 3H, O-arom), 7.4 (m, 5H, arom), 12.6 (s, 1H, NH).

Method (ii).

By Hydrolysis of 6-Substituted Benzyl-4-phenyl-3-methylmercapto-4,5-dihydro-1,2,4-triazin-5-ones **4b-d**.

A solution of each of compounds **4b-d** (0.5 g) in ethanol (10 ml) and concentrated hydrochloric acid (3 ml) was heated under reflux for one hour. After cooling, the precipitate obtained was collected and crystallized from acetic acid into colorless crystals of **9a-c** (*cf.* Table).

Compounds **9a-c** obtained from both methods i and ii have identical melting points (mixed melting points of identical compounds showed no depression). Identical compounds also had identical ir spectra.

6-Substituted Benzyl-4-amino-2,3,4,5-tetrahydro-1,2,4-triazin-3,5-diones **10a-c**.

Method (i).

From Arylpyruvic Acids **2b-d** and Carbohydrazide.

Each of the azlactones **1b-d** (0.1 mole) was hydrolysed as described before. After acidification with acetic acid, carbohydrazide (9.0 g) in boiling water (100 ml) was added with shaking. The reaction mixture was heated for 10 minutes and allowed to stand at room temperature for 3 hours. The precipitate obtained was collected and crystallized from ethanol into colorless crystals of **10a-c** (*cf.* Table).

Compound **10c**.

This compound had ir: ν max 3340, 3210 - 3160, 2960, 1685 - 1670, 1490, 1445, 1250, 920; ^{1}H-nmr (DMSO-d_6): δ 3.8 (s, 2H, CH_2-arom), 5.7 (s, 2H, NH_2), 6.0 (s, 2H, O-CH_2-O), 6.9 (m, 3H, arom), 12.7 (s, 1H, NH).

Method (ii).

By Hydrolysis of 6-Substituted Benzyl-4-amino-3-methylmercapto-4,5-dihydro-1,2,4-triazin-5-ones **6b-d**.

A solution of each of compounds **6b-d** (0.5 g) in ethanol (10 ml) and

concentrated hydrochloric acid (3 ml) was heated under reflux for 1 hour. After cooling, the precipitate obtained was collected and crystallized from ethanol into colorless crystals of **10a-c** (*cf.* Table).

Each of compounds **10a-c** obtained by method (ii) showed identical melting points to the corresponding compound obtained by method (i); melting points of a mixture of identical compounds obtained by both methods showed no depression. Identical ir spectra were obtained.

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