Synthesis and Evaluation of AChE Inhibitory Activity of 5,6-Diaryl-1,2,4-triazinyloxyacetyl-4-substituted Thiosemicarbazides, Triazoles and *N*-Benzylidene Derivatives

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A series of 1,2,4-triazinyl thiosemicarbazides, triazoles and *N*-benzylidene derivatives have been synthesised by condensation of 5,6-diphenyl-1,2,4-triazin-3-yloxyacetyl hydrazine with aromatic aldehydes and aryl isothiocyanates. Subsequent ring closure of thiosemicarbazides yielded the triazoles. All the compounds were subjected to *in vitro* testing of cholinesterase inhibitory action. Percentage inhibition was found to be moderate to good in a few of the compounds.

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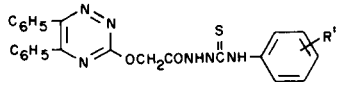
In the light of recent findings that 5,6-diphenyl-1,2,4-triazine nucleus is associated with diverse pharmacological activities such as antimicrobial (1), antiviral (2), anti-inflammatory (3-5), it was thought judicious to study the *in vitro* action of the synthesized triazine derivatives on acetylcholinesterase, a physiologically significant enzyme in the nervous system. It controls the hydrolysis of acetylcholine generated at the nerve junctions into choline. In the absence of effective acetylcholinesterase, the hydrolysis of acetylcholine to choline is prevented, consequently, there is an accumulation of excess acetylcholine at the synapse which leads to convulsions and death.

Chemistry.

Utilising the method of Blitz (6) 3-hydroxy-5,6-diphenyl-1,2,4-triazine (**1**) was obtained by refluxing an equimolar mixture of benzil and semicarbazide hydrochloride in acetic acid. Since the hydroxy group at position three was shown to have a phenolic nature (7) its esterification followed by hydrazinolysis was carried out smoothly to yield the targeted hydrazide **3**. Condensation of this hydrazide with suitable arylisothiocyanates resulted in the formation of 1-[(5,6-diphenyl-1,2,4-triazin-3-yl)oxyacetyl]-4-(*p*-substituted-phenyl)-3-thiosemicarbazides **4a-h**. These thiosemicarbazides on refluxing with 2*N* sodium hydroxide solu-

Table I

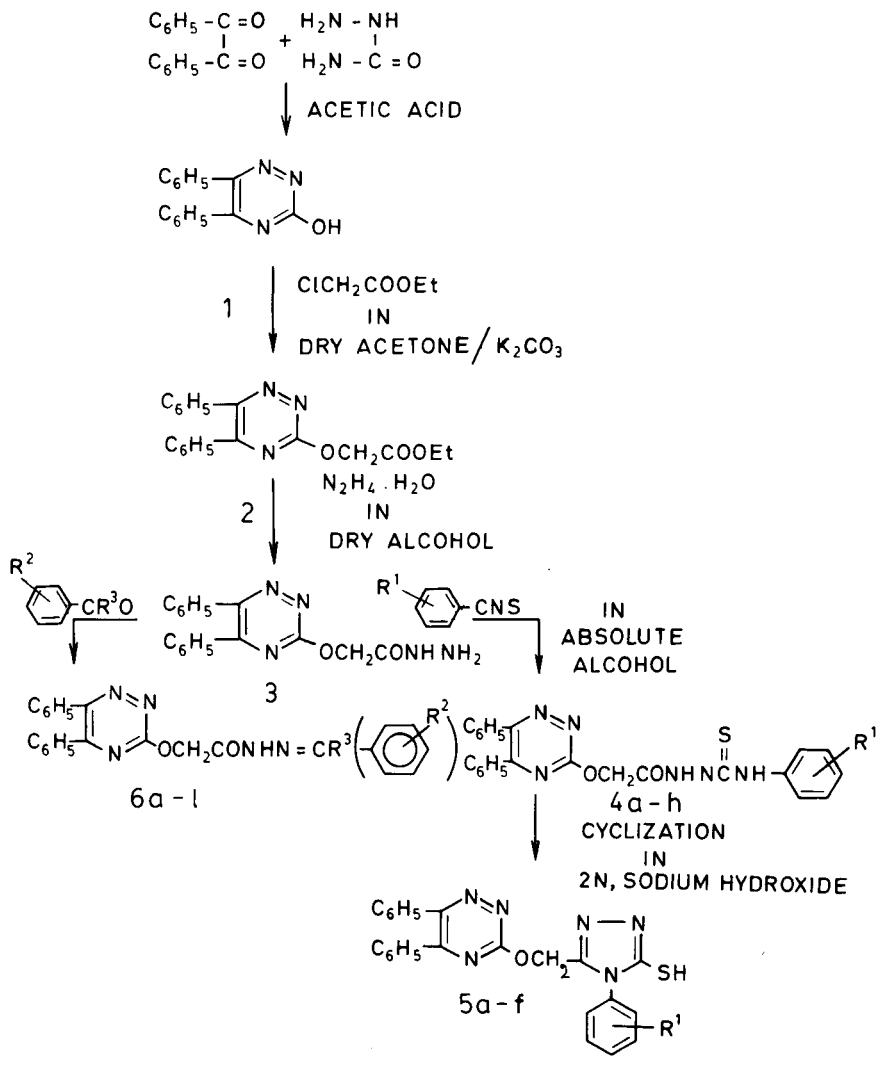
1-(5,6-Diphenyl-1,2,4-triazin-3-yl)oxyacetyl-4-(*p*-substituted-phenyl)-3-thiosemicarbazides **4a-h**



Compound No.	R'	Mp °C	Yield %	Molecular Formula		Analyses % C	H	N	% AChE Inhibition (a) 5×10^{-5} *M*
4a	H	195	68	$C_{24}H_{20}N_6O_2S$	Calcd.	63.1	4.3	18.4	31.82
					Found	62.8	4.4	18.1	
4b	3-CH_3	203	65	$C_{25}H_{22}N_6O_2S$	Calcd.	63.8	4.2	17.8	21.07
					Found	63.4	4.4	17.5	
4c	4-CH_3	201	60	$C_{25}H_{22}N_6O_2S$	Calcd.	63.8	4.2	17.2	22.0
					Found	63.5	4.5	17.5	
4d	3-Cl	212	62	$C_{24}H_{19}ClN_6O_2S$	Calcd.	58.7	3.8	17.1	25.07
					Found	58.4	3.8	17.2	
4e	4-Cl	198	70	$C_{24}H_{19}ClN_6O_2S$	Calcd.	58.7	3.8	17.1	49.55
					Found	58.5	3.9	17.0	
4f	4-OCH_3	200	66	$C_{25}H_{22}N_6O_3S$	Calcd.	61.7	4.5	17.2	21.37
					Found	61.8	4.3	17.1	
4g	4-OC_2H_5	205	68	$C_{26}H_{24}N_6O_3S$	Calcd.	62.4	4.8	16.8	20.46
					Found	62.6	4.6	16.6	
4h	4-Br	212	70	$C_{24}H_{19}BrN_6O_2S$	Calcd.	53.8	3.5	15.7	25.07
					Found	53.7	3.8	15.5	

(a) Propylene glycol was used as solvent.

Scheme - I



tion were cyclised into their corresponding 5-[(5,6-diphenyl-1,2,4-triazin-3-yl)oxyacetyl]-4-substituted-phenyl-4*H*-1,2,5-triazole-3-thiols **5a-f**.

Further the same hydrazide when subjected to simple aldol condensation with different aromatic aldehydes and keto compounds formed the *N*-benzylidene derivatives **6a-l**. The reaction sequence is shown by the Scheme I.

Acetylcholinesterase Inhibitory Activity.

Acetylcholinesterase inhibitory activity (8) was determined on rat-brain homogenate using acetylthiocholine as the substrate at 5×10^{-5} *M* concentration. The thiocholine formed by the enzymatic hydrolysis of acetylthiocholine was determined colorometrically. Decrease in thiocholine formation in experiments containing synthetic compounds was taken as an index of enzyme inhibition.

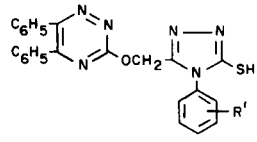
In the triazinyl thiosemicarbazides (**4a-h**) incorporation of substituents like methyl, methoxy, ethoxy and halogen groups on the phenyl ring of the thiosemicarbazide moiety, showed a decrease in AChE inhibitory activity as compared to the unsubstituted compound **4a**. However, compound **4h** possessing a chlorine atom exhibited enhanced inhibitory activity of 49.6%.

On cyclisation of thiosemicarbazides into triazoles, the various substituents increased the inhibition in the order methyl > methoxy > ethoxy > chloro. Compound **5c** having a bromo group showed the maximum inhibition of 70.0%.

Among the triazinyl *N*-benzylidene derivatives, maximum inhibition of 18.2% was observed in compounds **6a** and **6i**, whereas all the other compounds showed decreased inhibitory activity.

Table II

5-(5,6-Diphenyl-1,2,4-triazin-3-yl)oxymethyl-4-(-substituted-phenyl)-4*H*-1,2,4-triazole-3-thiols **5a-f**

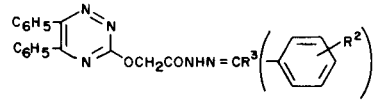


Compound No.	R¹	Mp °C	Yield %	Molecular Formula		Analyses% C	H	N	% AChE Inhibition (a) 5×10^{-5} *M*
5a	H	145	50	$C_{24}H_{18}N_6OS$	Calcd.	65.7	4.1	19.1	6.82
					Found	65.9	4.1	19.4	
5b	4-OCH_3	210	55	$C_{25}H_{20}N_6O_2S$	Calcd.	64.7	4.2	17.9	25.01
					Found	64.4	4.3	17.6	
5c	4-Br	125	58	$C_{24}H_{17}BrN_6OS$	Calcd.	55.7	3.2	16.2	70.5
					Found	55.5	2.9	16.2	
5d	4-OC_2H_5	127	52	$C_{26}H_{22}N_6O_2S$	Calcd.	64.7	4.5	17.4	23.64
					Found	64.4	4.4	17.2	
5e	4-Cl	182	56	$C_{24}H_{17}ClN_6OS$	Calcd.	61.0	3.6	17.7	21.6
					Found	61.1	3.8	17.6	
5f	4-CH_3	184	55	$C_{25}H_{20}N_6OS$	Calcd.	66.3	4.4	18.5	29.32
					Found	66.5	4.3	18.6	

(a) Propylene glycol was used as solvent.

Table III

(5,5-Diphenyltriazin-3-yl)oxyacetyl-*N*-(substituted-benzylidene) Hydrazines **6a-l**



Compound No.	R³	R²	Mp °C	Yield %	Molecular Formula		Analyses% C	H	N	% AChE Inhibition (a) 5×10^{-5} *M*
6a	H	4-OH	280	62	$C_{24}H_{19}N_5O_3$	Calcd.	67.7	4.4	16.4	18.19
						Found	67.5	4.0	16.1	
6b	H	4-Cl	200	66	$C_{24}H_{18}ClN_5O_2$	Calcd.	65.0	4.0	15.8	13.41
						Found	65.1	4.1	15.9	
6c	H	4-$N(CH_3)_2$	248	63	$C_{26}H_{24}N_6O_2$	Calcd.	69.0	4.5	18.5	9.1
						Found	68.8	4.5	18.5	
6d	H	2-naphthyl	259	55	$C_{28}H_{21}N_5O_2$	Calcd.	73.2	4.5	15.2	13.64
						Found	73.3	4.4	15.1	
6e	H	4-OH, 3-(OCH_3)	249	51	$C_{25}H_{21}N_5O_4$	Calcd.	65.0	4.9	15.8	14.78
						Found	65.2	5.0	15.6	
6f	H	2-furfural	223	56	$C_{22}H_{17}N_5O_3$	Calcd.	66.1	4.5	17.5	3.48
						Found	66.0	4.5	17.6	
6g	H	4-OCH_3	227	62	$C_{25}H_{21}N_5O_3$	Calcd.	68.3	4.8	16.0	3.57
						Found	68.4	4.6	16.2	
6h	CH_3	4-OCH_3	198	60	$C_{26}H_{23}N_5O_3$	Calcd.	68.8	5.0	15.4	10.23
						Found	68.9	5.1	15.5	
6i	H	3,4(OCH_3)$_2$	208	71	$C_{26}H_{23}N_5O_4$	Calcd.	66.5	4.9	15.0	18.26
						Found	66.3	4.7	14.8	
6j	—	2,3-indandione	212	62	$C_{25}H_{18}N_6O_3$	Calcd.	67.5	4.0	18.8	16.3
						Found	67.4	4.1	18.7	
6k	C_2H_5	4-Cl	227	64	$C_{26}H_{22}ClN_5O_2$	Calcd.	66.2	4.6	14.8	12.4
						Found	66.6	4.5	14.7	
6l	—	5-Cl, 2,3-indandione	202	58	$C_{25}H_{17}ClN_6O_3$	Calcd.	62.4	3.5	17.5	16.8
						Found	62.2	3.3	17.2	

(a) Propylene glycol was used as solvent.

EXPERIMENTAL

The melting points were obtained using an electrical melting point apparatus and are uncorrected. Infrared spectra (ν max cm^{-1}) were recorded on a Perkin-Elmer 137 spectrophotometer and the nmr spectra on a Varian EM-360 instrument using tetramethylsilane as internal reference with chemical shifts expressed in ppm. Purity of the compounds was checked on tlc plates using acetone-benzene medium and visualising the spots by exposure to iodine vapours.

(5,6-Diphenyl-1,2,4-triazin-3-yl)oxyacetate (**2**).

3-Hydroxy-5,6-diphenyl-1,2,4-triazine (24.9 g, 0.1 mole) and anhydrous potassium carbonate (20.7 g, 0.15 mole) were dissolved in an excess of acetone (dry) and heated for 30 minutes. Ethyl chloroacetate (14.2 ml, 0.1 mole) was added to the refluxing solution and the mixture heated for 48 hours. The excess solvent was removed and the residue was poured over crushed ice. A semi-solid separated out which solidified on standing overnight in the cold. It was filtered, dried and recrystallised from methanol giving 26 g (80%) of product, mp 150-152° dec; ir (potassium bromide): spectrum showed the appearance of a carbonyl band at 1690 cm^{-1} and the absence of an hydroxyl band at 3600 cm^{-1}; nmr (deuteriochloroform): δ 1.15-1.35 (t, 3H, CH_3), 4.05-4.3 (q, 2H, CH_2); 4.85 (s, 2H, OCH_2) and 7.1-7.4 (m, 10H, Ar-H).

Anal. Calcd. for $C_{19}H_{17}N_3O_3$: C, 68.45; H, 5.07; N, 12.53. Found: C, 68.43; H, 4.9; N, 12.60.

(5,6-Diphenyl-1,2,4-triazin-3-yl)oxyacetylhydrazide (**3**).

A solution of ethyl (5,6-diphenyl-1,2,4-triazin-3-yl)oxyacetate (16.8 g, 0.05 mole) and 99% hydrazine hydrate 3.5 ml (0.075 mole) in 100 ml of absolute ethanol was refluxed on a steam bath for 10 hours. The excess ethanol was removed under reduced pressure. The resultant solid obtained was filtered, dried and recrystallised from excess ethanol giving 10.5 g (62%) mp 184° dec; ir (potassium bromide): 1660 cm^{-1} (C=O), 3100 cm^{-1} (NH), 1620 cm^{-1} (CN), 3300 (NH_2); nmr (deuteriodimethylsulfoxide): δ 4.3-4.4 (s, 2H, OCH_2), 6.8-7.7 (m, 10H, Ar-H), 4.24 (d, 2H, NH_2), 9.4 (s, 1H, CON*H*).

Anal. Calcd. for $C_{17}H_{15}N_5O_2$: C, 63.5; H, 4.6; N, 21.8. Found: C, 63.39; H, 4.4; N, 21.82.

1-(5,6-Diphenyl-1,2,4-triazin-2-yl)oxyacetyl-4-(*p*-tolylphenyl)-3-thiosemicarbazides (**4c**), (**4a-h**) (Table I).

Equimolar quantities of (5,6-diphenyl-1,2,4-triazin-3-yl)oxyacetylhydrazide (0.963 g, 0.003 mole) and *p*-tolylisothiocyanate (0.45 ml, 0.003 mole) in 25 ml of absolute ethanol was refluxed on a steam bath for 3-5 hours. Excess ethanol was removed by distillation under reduced pressure. The crude product which separated out was filtered and washed several times with cold alcohol and petroleum ether, mp 201° dec, 0.7 g (60%); nmr (deuteriodimethylsulfoxide): δ 2.2 (s, 3H, CH_3), 3.3 (s, 2H, OCH_2), 6.9-7.2 (m, 14H, Ar-H), 8.1-8.9 (m, 3H, 3NH).

Anal. Calcd. for $C_{25}H_{22}N_6O_2S$: C, 63.3; H, 4.2; N, 17.2. Found: C, 63.5; H, 4.5; N, 17.5.

5-(4,6-Diphenyl-1,2,4-triazin-3-yl)oxymethyl-4-(4-ethoxyphenyl)-4*H*-1,2,4-triazole-3-thiol (**5d**), (**5a-f**) (Table II).

1-(5,6-Diphenyl-1,2,4-triazin-3-yl)oxyacetyl-4-(*p*-ethoxyphenyl)-3-thiosemicarbazide (1 g, 0.002 mole) was dissolved in 2*N* sodium hydroxide and the resulting solution was refluxed for 3 hours. It was cooled and filtered. The filtrate was acidified with dilute hydrochloric acid until complete precipitation occurred. It was filtered, washed with water and recrystallised from alcohol, mp 127° dec, 0.56 g (52%); nmr (deuteriochloroform): δ 1.1-1.5 (t, 3H, CH_3), 3.8-4.1 (m, 4H, 2 × CH_2), 6.8-7.8 (m, 14H, Ar-H).

Anal. Calcd. for $C_{26}H_{22}N_6O_2S$: C, 64.7; H, 4.5; N, 17.4. Found: C, 64.4; H, 4.4; N, 17.2.

(5,6-Diphenyl-1,2,4-triazin-3-yl)oxyacetyl-*N*-(4-chlorobenzylidene) Hydrazine (**6b**), (**6a-l**) (Table III).

To a solution of (5,6-diphenyl-1,2,4-triazin-3-yl)oxyacetylhydrazide (0.963 g, 0.003 mole) in 25 ml of absolute ethanol was added ethanolic solution of *p*-chlorobenzaldehyde (0.420 g, 0.003 mole) and the resulting mixture refluxed for 6 hours. The solid mass which separated on cooling was filtered and finally recrystallised from DMF/water, mp 200° dec, 0.84 g, (66%), ir (potassium bromide): 1690 cm^{-1} (CO), 1640 cm^{-1} (CN), 3100 cm^{-1} (NH); nmr (deuteriochloroform): δ 9.9 (m, 1H, CH), 8.55 (s, 1H, NH), 5.45 (s, 2H, CH_2), 7.2-7.85 (m, 14H, Ar-H).

Anal. Calcd. for $C_{24}H_{18}ClN_5O_2$: C, 65.0; H, 4.0; N, 15.8. Found: C, 65.1; H, 4.1; N, 15.9.

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