SUBSTANCES WITH ANTINEOPLASTIC ACTIVITY. XLVII.*

S-SUBSTITUTED 6-STYRYL-3-THIOXO-2,3,4,5-TETRAHYDRO-1,2,4-TRIAZIN-5-ONES AND ANALOGOUS 3-AMINO(HYDRAZINO) COMPOUNDS

M. BERAN, M. SEMONSKÝ and E. SVÁTEK

Research Institute of Pharmacy and Biochemistry, Prague 3

Received August 3rd, 1970

S-Substitution derivatives of 6-(3,4-methylenedioxystyryl)-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-5-one (*I*) *II*–*V* and *VII*, and analogous compounds *IX* and *X* were prepared. Proceeding from the ester *V*, or *VII*, 3-(4-carboxybutyl)thiotriazine *VI* and its 3-(5-carboxypentyl)thio analogue *VIII* were prepared. 3-Amino (*XI*) and 3-hydrazino (*XII*) analogue of triazine *I* and 3-arylidenehydrazino compounds *XIII* and *XIV* were also synthesized. The triazines *IV*—*VII* showed an antineoplastic effect on animals with some transplantable tumours.

In a previous paper[1] we took up the synthesis and the antineoplastic effect of some benzene-substituted 6-styryl-2,3,4,5-tetrahydro-1,2,4-triazin-3,5-diones and their 3-thioxo analogues. From the point of view of the effect studied the most interesting compound was 6-(3,4-methylenedioxystyryl)-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-5-one (*I*). In connection with studying the relationships between structure and antineoplastic effect of compounds we prepared now some S-substitution derivatives of *I*, compounds *II*—*VIII*, derived from its tautomeric 3-mercapto form and, for the purposes of comparison, also 6-styryl-3-methylthio-2,5-dihydro-1,2,4-triazin-5-one (*IX*) and the analogous 6-(3,4,5-trimethoxystyryl) compound *X* (Table I). With compounds *IV*—*VIII* it was assumed that they might have a cancerostatic effect as possible transport forms of triazine *I*, being better soluble in the lipid or aqueous components of the macroorganism than the very poorly soluble compound *I*. A favourable effect of the S-bound carboxyalkyl, especially carboxybutyl, group on the therapeutic index of antineoplastically active analogous S-substitution derivatives of 6-mercaptopurine was demonstrated before[2]. The effect of replacing the thioxo group of triazine *I* with an amino or hydrazino group was examined by preparing compounds *XI*—*XIV* (Table I).

* Part XLVI: This Journal *36*, 2254 (1971).

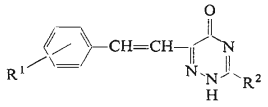
Compounds *II* and *III* were prepared by alkylation of triazine *I* with methyl and ethyl iodide, respectively, in sodium hydroxide[3] and, using the same procedure with the corresponding 6-styryl-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-5-ones (ref.[1]) we obtained the methylthiotriazines *IX* and *X*. Compounds *IV*, *V* and *VII* were prepared by a reaction of triazine *I* with ethyl or n-butyl δ-bromovalerate, or with ethyl ω-bromocaproate, at a higher temperature in the presence of dimethylformamide and potassium carbonate. Proceeding from esters *V* and *VII* we obtained the corresponding acids *VI* and *VIII* by alkaline hydrolysis conducted at room temperature. Reaction of the S-ethylthio compound *III* with methanolic ammonia carried out in a sealed tube at 110°C yielded the 3-amino compound *XI*. For purposes of comparison, compound *XI* was also prepared by boiling the guanylhydrazone of 3,4-methylenedioxybenzylidenepyruvic acid or its potassium salt, in aqueous potassium carbonate, in analogy with the procedure described in ref.[4] (for the cyclization procedure used with guanylhydrazone see also ref.[5]). The hydrazinolysis of methylthiotriazine *II* carried out in ethanol at the boiling temperature of the reaction mixture, yielded the 3-hydrazino compound *XII* from which, by boiling with benzaldehyde or with its 4-methoxy derivative, in dimethylformamide we obtained the 3-benzylidenehydrazino or 3-(4-methoxybenzylidene)hydrazino compound *XIII* or *XIV*.

When examining the IR spectra we observed that compounds *IV* and *VII* in a dioxane solution (the other compounds are not soluble in dioxane) show a marked absorption at 1673 cm^{-1} which is typical of the lactam carbonyl, or of the presence of the two compounds in a tautomeric lactim from. The carbonyl group of compounds studied in the solid state (in a KBr pellet or in Nujol) shows absorption at lower frequencies in the region of about 1600 cm^{-1}. This shift toward lower frequencies is apparently due to the presence of hydrogen bonds in the solid state. The IR spectrum of the triazine *XI* permits the conclusion on the basis of the work of Gut and coworkers[6,7] that the triazine in question has the structure of 6-(3,4-methylenedioxystyryl)-3-amino-2,5-dihydro-1,2,4-triazin-5-one.

An informative evaluation of the compounds as to their antineoplastic effect on H strain mice and Wistar rats with transplantable tumours was carried out at this institute by Dr V. Jelínek with coworkers (H-strain mice: Crocker's sarcome 180—S 180; originally methylacridine-induced sarcome — Sak; adenocarcinome of the mammary gland — HK; S 37 sarcome. Rats: Yoshida ascitic sarcome — Y. The technique used in the evaluation of the effect is described in ref.[8]). From the point of view of the antineoplastic effect, the triazines *IV*—*VIII* were most interesting. The compounds were applied *per os* in a daily dose of 200 mg/kg beginning on the 3rd day (with S 180, Sak, KH), or on the 2nd day (with S 37 and Y) after transplantation of the tumour, continuously for 12 days, with the exception of the Y tumour animals where the application lasted only 5 days. Animals with the Sak tumour responded favourably to *V* and *VII*: In comparison with the control group of animals the treated animals survived by 25% and 14% longer with a simultaneous suppression of tumour growth by 38 and 35%, respectively. (In this consideration, 100% = lifespan, tumour size of the control group.) In animals with the S 180 tumour, *IV* and *V* and *VII* suppressed the tumour growth by 21, 19 and 28%, respectively, without any appreciable

TABLE I

3-Substituted 6-Styryl-2,5-dihydro-1,2,4-triazin-5-ones



I, $R^1 = 3,4\text{-}CH_2O_2$, $R^2 = SH$
II–*VIII*, *XI*–*XIV*, $R^1 = 3,4\text{-}CH_2O_2$
IX, $R^1 = H$
X, $R^1 = 3,4,5\text{-}(CH_3O)_3$

Compound R^2	M.p., °C (solvent)	Yield %	Formula (M.w.)	Calculated / Found % C	% H	% N	% S	UV-spectrum[a] 0,1M-HCl λ_{max}, nm (log ε)	0,1M-NaOH λ_{max}, nm (log ε)	IR spectrum[b] cm^{-1}
II S-CH_3	248–250 (ethanol)	58	$C_{13}H_{11}N_3O_3S$ (289·3)	53·96 / 53·93	3·83 / 4·24	14·52 / 14·70	11·08 / 11·14	240 (4·28) 364 (4·41)	225 (4·35) 282 (3·96) 363 (4·47)	997, 1 504, 1 560 1 585, 1 608, 1 617
III S-C_2H_5	231–232 (ethanol)	60	$C_{14}H_{13}N_3O_3S$ (303·3)	55·43 / 55·39	4·31 / 4·58	13·86 / 13·60	10·57 / 10·46	241 (4·29) 364 (4·44)	226 (4·35) 282 (3·98) 364 (4·48)	976, 1 492, 1 505 1 560–1 590w, 1 604 1 620
IV S-$(CH_2)_4CO_2C_2H_5$	167–168 (methanol)	14	$C_{19}H_{21}N_3O_5S$ (403·5)	56·55 / 56·26	5·24 / 5·18	10·41 / 10·31	7·94 / 7·87	241 (4·36) 366 (4·53)	228 (4·41) 365 (4·55)	974, 1 577, 1 598 1 620, 1 732
V S-$(CH_2)_4CO_2$-n-C_4H_9	163–164 (benzene)	21	$C_{21}H_{25}N_3O_5S$ (431·5)	58·45 / 58·63	5·84 / 5·91	9·74 / 9·71	7·43 / 7·54	242 (4·29) 363 (4·43)	284 (4·01) 366 (4·49)	986, 1 509, 1 594 1 736
VI S-$(CH_2)_4CO_2H$	227–228 (methanol)	70	$C_{18}H_{17}N_3O_5S$ (387·4)	55·80 / 55·53	4·42 / 4·70	10·84 / 11·05	8·27 / 8·57	241 (4·26) 365 (4·43)	229 (4·31) 282 (3·98) 365 (4·45)	986, 1 504, 1 592 1 690
VII S-$(CH_2)_5CO_2C_2H_5$	169–170 (benzene)	?	$C_{20}H_{23}N_3O_5S$ (417·5)	57·53 / 57·25	5·55 / 5·49	10·06 / 10·33	7·68 / 7·91	241 (4·15) 299 (4·07) 367 (4·39)	222 (4·30) 282 (4·06) 362 (4·43)	987, 1 572, 1 609 1 739

VIII S-$(CH_2)_5CO_2H$	223–224 (methanol)	82	$C_{18}H_{19}N_3O_5S$ (389·4)	55·51 55·41	4·92 4·98	10·78 10·54	8·23 8·42	208 (4·23) 239 (4·15) 362 (4·37)	224 (4·28) 280 (4·02) 360 (4·44)	979, 1 551, 1 600 1 620, 1 631, 1 695 1 747
IX S-CH_3	246–247 (ethanol)	79	$C_{12}H_{11}N_3OS$ (245·3)	58·75 58·97	4·52 4·93	17·13 17·06	13·07 13·00	240 (4·28) 273 (4·01) 339 (4·42)	229 (4·31) 275 (4·12) 353 (4·48)	978, 1 495, 1 503 1 573, 1 622
X S-CH_3	209–210 (methanol)	52	$C_{15}H_{17}N_3O_4S$ (335·4)	53·72 53·89	5·11 5·34	12·53 12·69	9·56 9·42	217 (4·39) 241 (4·35) 353 (4·42)	225 (4·42) 284 (4·01) 359 (4·42)	984, 1 508, 1 580 1 622
XI NH_2	>350 (acetic acid)	31	$C_{12}H_{10}N_4O_3$ (258·2)	55·82 55·53	3·90 4·17	21·70 21·74	—	279 (4·02) 363 (4·27)	279 (3·97) 358 (4·37)	969, 1 503, 1 532 1 600, 1 630, 1 660 1 696, 3 360 w
XII $NHNH_2$	>350 (aqueous dimethyl- formamide)	15	$C_{12}H_{11}N_5O_3$ (273·3)	·	— —	25·64 25·60	— —	282 (4·01) 368 (4·19)	220 (4·15) 285 (4·01) 361 (4·26)	981, 1 503, 1 615
XIII $NHN{=}CHC_6H_5$	290–291 (dimethyl- formamide)	33	$C_{19}H_{15}N_5O_3$ (361·4)		— —	19·38 19·06	— —	309 (4·41) 375 (4·52)	225 (4·37) 271i(4·26) 360 (4·61)	972, 1 503, 1 558 1 620
XIV $NHN{=}CHC_6H_4OCH_3$ -*p*	292–295 (dimethyl- formamide)	40	$C_{20}H_{17}N_5O_4$ (391·4)	61·39 60·97	4·36 4·74	17·89 17·72	— —	301 (4·44) 367 (4·50)	220 (4·35) 275 (4·29) 366 (4·63)	970, 1 504, 1 560 1 625

[a] UV spectra in 50% ethanol. [b] IR spectra measured in a KBr pellet or in Nujol. The IR spectrum of compounds in a dioxane solution (cm^{-1}): *IV*: 1 673, 1 747; *VII*: 1 540, 1 582, 1 608, 1 630, 1 673, 1 705i, 1 720i, 1 745; w wide band, i inflexion.

effect on the survival of the treated animals. Compound *VIII* brought about a 15% longer survival of animals with the S 37 tumour and inhibited its growth by 24%. At a dose of 400 mg/kg, under otherwise identical conditions, the animals survived by 31% longer, the tumour growth being inhibited by 27%. In the case of animals with the HK tumour triazine *IV* suppressed tumour growth by 33% and triazine *VI* by 25% without any significant effect on the survival of the experimental animals. Rats with the Y tumour where only the survival of the animals could be examined, only compound *VI* resulted in a 22% greater survival time.

EXPERIMENTAL

The melting points or the point of decomposition of the compounds were determined in a Kofler block and are not corrected. Compounds for analysis were dried, unless stated otherwise, at 100°C and at 0·5 Torr. The IR spectra of the compounds were recorded in a Unicam SP 200 G spectrophotometer, the UV spectra on a SP 700 spectrophotometer in a 1 cm silica cuvette (Table I).

6-(3,4-Methylenedioxystyryl)-3-methylthio-2,5-dihydro-1,2,4-triazin-5-one (*II*) and Analogous Compounds *III*, *IX* and *X*

A mixture of a solution of triazine *I*, or of its 6-styryl- and 6-(3,4,5-trimethoxystyryl) analogue[1] (0·01 mol) in 25 ml 1M-NaOH and 0·011 mol methyl iodide or ethyl iodide in the case of preparation of *III*, was shaken for 10 min and left to stand for 30 min at room temperature. After acidification with hydrochloric acid to pH 1 and leaving to stand overnight at 5°C, the crude product was filtered, washed with water and purified by crystallization (Table I).

6-(3,4-Methylenedioxystyryl)-3-(4-ethoxycarbonylbutyl)-thio-2,5-dihydro-1,2,4-triazin-5-one (*IV*) and Triazines *V* and *VII*

A mixture of a warm solution of 27·5 g (0·1 mol) triazine *I* in 600 ml dimethylformamide, 15 g calcined potassium carbonate and 0·1 mol ethyl or n-butyl δ-bromovalerate (for compound *IV*, *V*) or ethyl ester of ω-bromocaproic acid (for compound *VII*) was heated for 2·5 h (*IV*, *V*) or for 5 h (*VII*) on a boiling water bath in the absence of air moisture. The mixture was filtered, the volatile components were removed by distillation in water-pump vacuum, the residue was extracted with 350 ml hot benzene and the extract was diluted with 250 ml n-hexane. By standing overnight at 5°C, a solid product precipitated and was purified by crystallization (Table I). From the strongly viscous fraction which precipitated together with the solid, another fraction of the product was isolated after dissolving in benzene, filtration and dilution of the filtrate with light petroleum.

6-(3,4-Methylenedioxystyryl)-3-(4-carboxybutyl)thio-2,5-dihydro-1,2,4-triazin-5-one (*VI*) and Triazine *VIII*

A mixture of 0·01 mol ester *V* or ester *VII* with 75 ml 0·4M-NaOH was left to stand under occasional shaking for 48 h at 20°C. The filtered solution was made acid with 5% sulfuric acid to pH 2 and the product precipitated was purified by crystallization (Table I).

6-(3,4-Methylenedioxystyryl)-3-amino-2,5-dihydro-1,2,4-triazin-5-one (*XI*)

a) 690 mg guanyl hydrazone of 3,4-methylenedioxybenzylidenepyruvic acid was dissolved in 90 ml boiling water with an addition of 1 g potassium carbonate and the mixture was refluxed for 30 min. The product (640 mg) precipitated after acidification of the mixture with hydrochloric acid to pH 2, was boiled twice with 20 ml ethanol and further purified by crystallization (Table I).

The required guanyl hydrazone was obtained by acidification of a mixture of 1·36 g aminoguanidine carbonate and 10 ml water with hydrochloric acid and by mixing the solution with a warm solution of 2·2 g 3,4-methylenedioxybenzylidenepyruvic acid[1] in a mixture of 45 ml ethanol and 20 ml water. After 20 h of standing of the mixture at 20°C the precipitated product (1·23 g) was filtered and purified by repeated boiling with ethanol. The product obtained (1·1 g) does not melt below 350°C. For $C_{12}H_{12}N_4O_4$ (276·3) calculated: 52·16% C, 4·38% H, 20·28% N; found: 52·08% C, 4·55% H, 20·03% N.

b) A mixture of 500 mg triazine *III* and 5 g methanolic solution of ammonia containing 0·85 g NH_3 was heated for 4 h in a sealed tube at 110°C. After cooling of the mixture to 5°C the product (160 mg) was purified in the same way as shown above. On heating, the substance did not melt below 350°C. During paper chromatography using n-butanol–acetic acid–water (4 : 1 : 5) and n-butanol saturated with 1·5M ammonia (detected with UV light at 254 nm) the preparation showed the same properties as the compound prepared according to procedure (*a*). For $C_{12}H_{10}N_4O_3$ (258·2) calculated: 55·82% C, 3·90% H, 21·70% N; found: 55·91% C, 3·80% H, 21·87% N.

6-(3,4-Methylenedioxystyryl)-3-hydrazino-2,5-dihydro-1,2,4-triazino-5-one (*XII*) and Compounds *XIII* and *XIV*

A mixture of 1 g triazine *II*, 500 ml ethanol and 5 ml hydrazine hydrate was refluxed for 4 h, the volatile fractions were removed by distillation, the residue boiled with ethanol, dissolved by heating in dimethylformamide and the hot solution was diluted with water. The precipitated product (200 mg) was purified by crystallization (Table I). A mixture of 680 mg triazine *XII* with 270 mg benzaldehyde or 340 mg *p*-anisaldehyde, and 12 ml dimethylformamide was refluxed for 15 min and left to stand overnight at 5°C. The precipitated product *XIII* or *XIV* was purified by crystallization (Table I).

The analyses were done by Mr K. Havel and Mrs M. Komancová (direction by Dr J. Kőrbl) in the analytical department of our Institute; the UV spectra were recorded by Dr J. Vachek and paper chromatography was done by Mrs M. Jelínková and Dr K. Macek of our Institute.

REFERENCES

1. Semonský M., Beran M., Neumannová J., Škvorová H., Jelínek V.: This Journal *32*, 4439 (1967).
2. Semonský M., Černý A., Jelínek V.: This Journal *25*, 1091 (1956).
3. Barlow R. B., Welch A. D.: J. Am. Chem. Soc. *78*, 1258 (1956).
4. Miura K., Ikeda M., Kondo T., Setogawa K.: Kanazawa Daigaku Yakugakubu Kenkyu Nempo *11*, 25 (1961); Chem. Abstr. *56*, 4767 (1962).
5. Gut J., Hesoun D., Nováček A.: This Journal *31*, 2014 (1966).
6. Gut J., Jonáš J., Pitha J.: This Journal *29*, 1394 (1964).
7. Pitha J., Fiedler P., Gut J.: This Journal *31*, 1864 (1966).
8. Jelínek V.: Neoplasma *7*, 146 (1960).

Translated by A. Kotyk.