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Synthesis of Some Substituted Triazolo[4,3-b][1,2,4]triazines as Potential Anticancer Agents

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The synthesis of some 3-substituted amino-6,7-diphenyl-1,2,4-triazolo[4,3-b][1,2,4]triazines (**15**) by cyclodesulfurisation of the corresponding N-(5,6-diphenyl-1,2,4-triazin-3-yl)-N′-[substituted thio (carbamoyl)]hydrazines (**3**) using dicyclohexylcarbodiimid (*DCC*) and mercuric chloride is described. Moreover, trials to prepare 3-substituted amino-7-hydroxy-6-methyl-1,2,4-triazolo[4,3-b][1,2,4]triazines were not successful.

(Keywords: Antineoplastic; Potential-azapurine analogues; Triazolo[4,3-b][1,2,4]triazine, synthesis and biological evaluation)

Synthese einiger substituierter Triazolo[4,3-b][1,2,4]-triazine als potentielle Antikrebswirkstoffe

Es wird die Synthese einiger 3-substituierter Amino-6,7-diphenyl-1,2,4-triazolo[4,3-b][1,2,4]-triazine (**15**) mittels Cyclodesulfurisierung der entsprechenden N-(5,6-Diphenyl-1,2,4-triazin-3-yl)-N′-[subst.thio(carbamoyl)]-hydrazine (**3**) unter Verwendung von Dicyclohexylcarbodiimid (*DDQ*) beschrieben. Versuche zur Herstellung von 3-substituierten Amino-7-hydroxy-6-methyl-triazolo[4,3-b][1,2,4]-triazinen schlugen fehl.

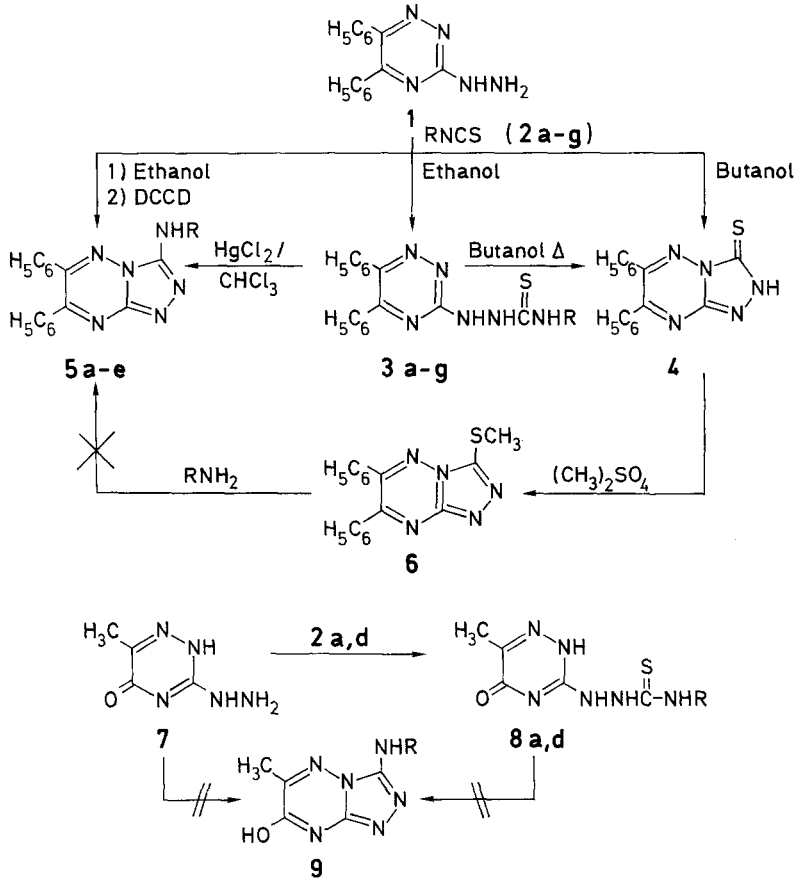
Introduction

Structural modification of purine bases is one of the most important approaches for designing new anticancer and antiviral agents. This led to the development of some aza-analogues which are potent purine antagonists [1–3]. Recently we have reported the synthesis and antineoplastic properties of some imidazo[1,2-b][1,2,4]triazines and pyrimido[1,2-b][1,2,4]triazines [4] which are structurally related to natural and synthetic purines. As a continuation of our work dealing with the synthesis and antineoplastic properties of several heterocyclic systems comprising 1,2,4-triazine nucleus [5–7], we now report the synthesis and

antileukemic activity of some 3-substituted amino-6,7-diphenyl-1,2,4-triazolo[4,3-b][1,2,4]triazines (**5 a–e**) and their key intermediates N-(5,6-diphenyl-1,2,4-triazin-3-yl)-N′-[substituted thio (carbamoyl)]hydrazines (**3 a–g**).

Results and Discussion

Reacting equimolecular quantities of the hydrazine [8] (**1**) and the pertinent isothiocyanate (**2 a–g**) in boiling ethanol yielded the corresponding N-(5,6-diphenyl-1,2,4-triazin-3-yl)-N′-[alkyl or arylthio (carbamoyl)]hydrazines (**3 a–g**, Table 1). On the other hand, reacting **1** with any of **2 a–g** in boiling butanol afforded, unexpectedly, a single



R = **a**: C_6H_5—; **b**: *m*-$CH_3 \cdot C_6H_4$—; **c**: *p*-$Cl \cdot C_6H_4$—; **d**: *p*-$Br \cdot C_6H_4$—; **e**: $C_6H_5 \cdot CH_2$—; **f**: cyclo-C_6H_{11}—; **g**: C_4H_9—

Table 1. *N-(5,6-diphenyl-1,2,4-triazin-3-yl)-N'-[substituted thio(carbamoyl)]-hydrazines* (**3a–g**) *and 3-substituted amino-6,7-diphenyl-triazolo[4,3-b][1,2,4]-triazines* (**5a–e**)

No.	Yield %	M.p. °C	Molecular formula	Analysis (%), calcd./found			
				C	H	N	S
3a	92	173–175	$C_{22}H_{18}N_6S$ 398.48	66.31 66.30	4.55 4.30	21.09 21.10	8.05 8.30
3b	90	169–171	$C_{23}H_{20}N_6S$ 412.50	66.96 67.20	4.89 5.10	20.38 20.30	7.77 7.30
3c	82	151–153	$C_{22}H_{17}ClN_6S$ 432.93	61.03 61.30	3.96 4.30	19.41 19.10	7.41 7.30
3d	93	161–162	$C_{22}H_{17}BrN_6S$ 477.39	55.35 55.70	3.59 3.60	17.61 17.50	6.72 6.50
3e	88	179–180	$C_{23}H_{20}N_6S$ 412.50	66.96 66.60	4.89 4.60	20.38 20.10	7.77 7.70
3f	85	205–207	$C_{22}H_{24}N_6S$ 404.53	65.32 65.32	5.98 5.80	20.78 20.60	7.93 8.00
3g	80	188–190	$C_{20}H_{22}N_6S$ 378.50	63.46 63.80	5.86 5.80	22.21 22.21	8.47 8.80
5a	85	220–221	$C_{22}H_{16}N_6$ 364.39	72.51 72.60	4.43 4.40	23.07 23.10	
5b	75	182–183	$C_{23}H_{18}N_6$ 378.42	73.00 73.20	4.80 4.60	22.20 22.40	
5c	60	243–244	$C_{22}H_{15}ClN_6$ 398.85	66.25 66.20	3.79 3.90	21.07 21.10	
5d	62	228–229	$C_{22}H_{15}BrN_6$ 443.30	59.60 60.00	3.41 3.10	18.96 18.90	
5e	49	184–185	$C_{23}H_{18}N_6$ 378.42	73.00 73.20	4.80 5.00	22.20 22.40	

product. The isolated product was proved to be identical in every respect with 3-mercapto-6,7-diphenyl-1,2,4-triazolo[4,3-b][1,2,4]triazine (**4**). The latter, **4** was unequivocally prepared following the reported procedure [7]. Mechanistically, the formation of the bicyclic compound **4** from **1** and **2a–g** in refluxing butanol involves the initial formation of **3** which undergoes immediate intramolecular nucleophilic attack of ring-2 nitrogen on the thione group with elemination of the substituted amino group. This mechanism was proved by converting any of **3a–d** to **4** upon boiling in butanol. However, the isolation of **3a–g** from the reaction of **1** and **2a–g**

in boiling ethanol indicates that **3 a–g** are thermostable at the boiling point of ethanol.

The synthesis of 3-substituted amino-6,7-diphenyl-1,2,4-triazolo[4,3-b][1,2,4]triazines (**5 a–e**, Table 1) was accomplished by the application of two different methods of cyclodesulfurisation [10]. In the first method, a mixture of equimolar amounts of **1** and **2 a–g** was refluxed for 30 min, followed by treatment with 1.5 mol *DCC*. Whereas, in the second one, the cyclodesulfurisation was achieved by reacting **3 a–e** with mercuric chloride in boiling chloroform.

The cyclisation proceeds smoothly with aryl or aralkyl isothiocyanates, but failed with butyl or cyclohexyl isothiocyanates.

Attempts to prepare **5 a–e** by reacting **6** [9] with different primary amines either in high boiling solvents or by fusion were unsuccessful.

Analogously, N-(6-methyl-2,5-dihydro-5-oxo-1,2,4-triazin-3-yl)-N′-[arylthio (carbamoyl)]hydrazines (**8 a, d**) were prepared by reacting the hydrazine [11] (**7**) with **2 a, d**. However, the cyclodesulfurisation of the latter compounds to **9** by the two methods was unsuccessful. This might by attributed to the electron attracting ability of carbonyl group at position-5 which would destabilize the fused ring system.

Antineoplastic Screening

Compounds **3 b, d, e** and **5 b–e** were screened against P388 lymphotic leukemia according to a standard protocol*. Median survival time was taken as the activity parameter for tumor evaluation. A compound is considered active if the ratio of median survival time for treated to control mice (T/C) is $\geqslant 120\%$. None of the compounds did reach this value when tested by a three dose assay at 400, 200 and 100 mg/kg body weight. However, the results indicate that compounds **3 b, d, e** are toxic while the cyclised compounds **5 b–e** are non-toxic and have higher T/C values.

Experimental

Melting points are uncorrected. IR spectra: Beckman 4210. ^{1}H NMR spectra: Varian EM 360 with *TMS* as internal standard.

N-(5,6-diphenyl-1,2,4-triazin-3-yl)-N′-[aryl and alkyl thio(carbamoyl)] hydrazines (**3 a–g**)

A solution of equimolar amounts of **1** and the appropriate **2 a–g** (5 mmol of each) in ethanol (40 ml) was refluxed for 30 min. The precipitated product was filtered and recrystallised from chloroform—ethanol. IR (nujol): 3 450–3 050 (NH), 1 635–1 575 (C=N and C=C), 1 555–1 520 cm^{-1}. ^{1}H NMR ($CDCl_3$) of **3 b**: δ(ppm) = 2.3 (s, 3 H, CH_3), 6.8–7.6 (m, 14 H, *Ar*-H), 9.8–10.08 (m, 3 H, 3 NH); for **3 c** (*DMSO-d*$_6$): δ(ppm) = 7.2–7.8 (m, 14 H, *Ar*-H), 9.89–10.12 (m, 3 H, 3 NH).

* Conducted by the National Cancer Institute, Bethesda, Maryland, U.S.A.

3-Mercapto-6,7-diphenyl-1,2,4-triazolo[4,3-b][1,2,4]triazine (**4**)

Method A: Preparation by reacting **1** with carbon disulfide and potassium hydroxide following [7].

Method B: A solution of equimolar quantities of **1** and any of **2a–g** (2 mmol of each) in butanol (20 ml) was refluxed for 2 h. Upon cooling the precipitated product was filtered and crystallised from *DMF*, m.p. 298–300 °C. It was identical in every respect with that prepared by method A.

Method C: Preparation in almost quantitative yield by refluxing a solution of any of **3a–g** in butanol (20 ml) for 2 h. The cooled reaction mixture was then worked up as described under method B.

3-Substituted amino-6,7-diphenyl-1,2,4-triazolo[4,3-b][1,2,4]triazines (**5a–e**)

Method A: A solution of **1** (0.8 g, 3 mmol) and the proper **2a–e** (3 mmol) was heated in ethanol under reflux for 30 min. *DCC* (0.95 g, 4.6 mmol) was added and heated under reflux for 10 h. Most of ethanol was removed and the residue crystallised from ethanol or chloroform—ethanol.

Method B: A mixture of the proper **3a–e** (1.38 mmol) and mercuric chloride (1.12 g, 4.13 mmol) in dry chloroform (50 ml) was heated under reflux for 18 h. Chloroform was evaporated and the residue boiled in ethanol (25 ml) containing 20% aqueous hydrochloric acid solution (30 ml) and saturated with hydrogen sulfide gas. The mercuric sulfide formed was filtered, and the filtrate evaporated until all ethanol was removed. The aqueous acidic solution was made distinctly alkaline with sodium hydrogen carbonate to give the desired products. IR (nujol): 3 350–3 200 (NH), 1 615, 1 590–1 560 (C=N and C=C) cm^{-1}. ^1H NMR (*DMSO-d_6*) of **5b**: δ(ppm) = 2.33 (s, 3 H, CH_3), 7.35–7.65 (m, 14 H, *Ar*-H), 8.03 (s, 1 H, NH); for **5c** (*DMSO-d_6*): δ(ppm) = 7.1–7.8 (m, 14 H, *Ar*-H), 10.48 (s, 1 H, NH).

N-(6-methyl-2,5-dihydro-5-oxo-1,2,4-triazin-3-yl)-N'-[phenyl-thio(carbamoyl)]hydrazine (**8a**)

A solution containing equimolecular amounts of **7** and **2a** (10 mmol of each) in ethanol (20 ml) was refluxed for 2 h. The alcohol was then removed and the residue crystallised from ethanol as yellow crystals m.p. 187–189 °C, yield 270 mg (98%). IR (nujol): 3 500–3 200 (NH), 1 675 (C=O), 1 630–1565 (C=N and C=C), 1 540–1 520 cm^{-1}.

$C_{11}H_{12}N_6OS$ (276.32). Calcd.: C 47.81 H 4.38 N 30.42.
Found: C 47.60 H 4.40 N 30.30.

N-(6-methyl-2,5-dihydro-5-oxo-1,2,4-triazin-3-yl)-N-[p-bromophenyl-thio (carbamoyl)]hydrazine (**8d**)

This was similarly prepared from **7** and **2d** (10 mmol of each) and recrystallised from ethanol as pale yellow crystals m.p. 192–193 °C.

$C_{11}H_{11}BrN_6OS$ (355.29). Calcd.: C 37.19 H 3.12 N 23.66.
Found: C 37.20 H 3.30 N 23.40.

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