SYNTHESIS OF NOVEL 5-MERCAPTO-s-TRIAZOLO[3,4-c]-as-TRIAZINO[5,6-b]INDOLES AND THEIR MANNICH BASES +

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Abstract --- Reaction of as-triazino[5,6-b]indol-3-ylhydrazines with carbon disulphide in the presence of methanolic potassium hydroxide gave the title compounds through angular cyclization. On subjecting the title compounds to Mannich reaction, the NH of the triazole ring underwent aminomethylation reaction. This is further confirmed by chemical routes and by recording the uv spectra of linearly and angularly cyclized products. Structures of all the newly synthesized compounds are established on the basis of elemental analysis, ^{1}H nmr and mass spectral data. Few selected compounds are screened for their antiviral and antibacterial properties.

The importance of indole nucleus is well established in pharmaceutical chemistry, as many of its derivatives are used as antipyretic, anticonvulsant, analgesic and antidepressant agents.[1-3] This has given an impetus for the synthesis of several triazinoindoles. Some of these triazinoindoles are now considered as potential drugs for the treatment of common cold infections caused by different rhino virus strains.[4-7] These compounds were active in vitro not only against rhino viruses but also against Coxsackie, Echo, herpes, polio, pseudorabies and vaccinia viruses.[5] The clinical efficacy of as-triazines [8] and methisazone[9] prompted us to prepare a series of 3-hydrazinoindolotriazines and their cyclization products.

The intermediate 3-hydrazino-as-triazino[5,6-b]indoles (**3**) were prepared by the reaction of isatins with thiosemicarbazide , followed by hydrazinolysis as reported earlier.[10] These 3-hydrazinoindolotriazines (**3**), on treatment with carbon disulphide in the presence of methanolic potassium hydroxide, yielded the title compounds (**4a-e**) after usual work up. From the analytical and spectral data, structures (**4**) are now given to these compounds as shown in Scheme 1

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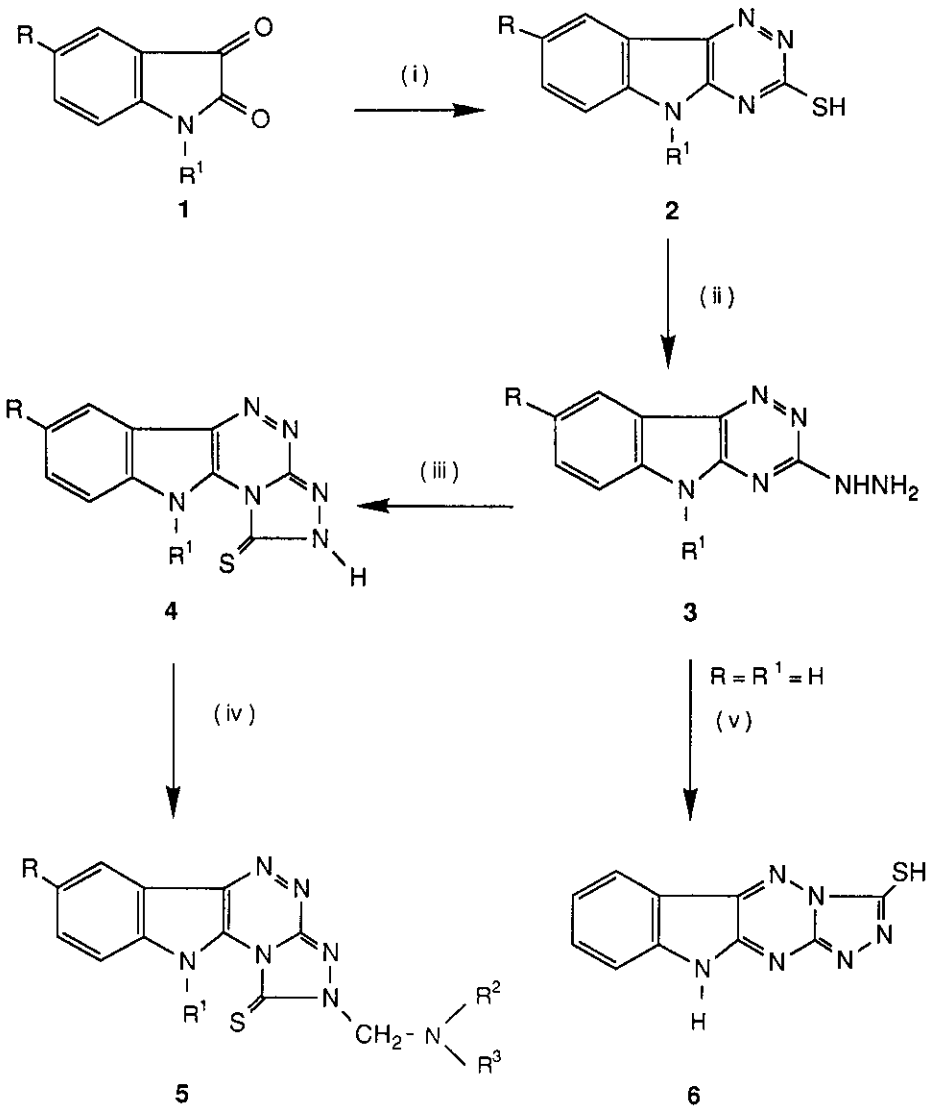


(i) $NH_2NH\,CS\,NH_2$, K_2CO_3 / H_2O (ii) $N_2H_4 . H_2O$

(iii) CS_2 / KOH, MeOH (iv) HCHO, $HN(R^2)(R^3)$ / DMF

(v) CS_2 / Pyridine

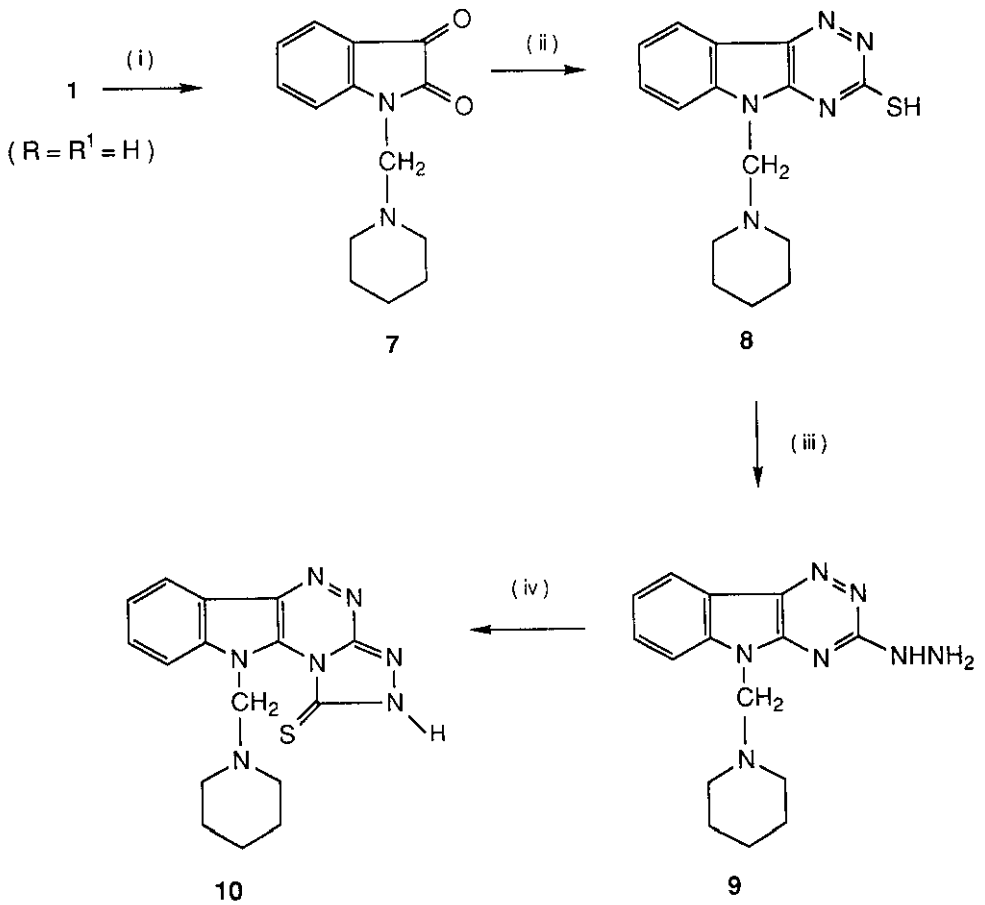
Scheme 1

That the reaction of hydrazines (**3**) yielded products (**4**) through angular cyclization instead of the known linear cyclization is proved as follows . When our work was in progress, we found that Ram et al.[11] carried out the cyclization of hydrazines (**3**) using pyridine as a catalyst and reported the formation of the linearly cyclized product (**6**). Our cyclization product (**4**) was distinctly different from (**6**). The mass spectrum of our cyclized product (**4a**) showed a molecular ion peak at m/z, 242 consistent with the assigned structure and the compound did not melt up to 350^0C, while the linearly cyclized product (**6**) had a mp 295^0C. Our product (**4a**) was dark red in colour, while (**6**) was brownish . The uv spectra of these two cyclization products were also distinctly different. The uv spectrum of compound (**4a**) had peaks at λ_{max} 306, 343, 378 and 479 nm, while that of (**6**) had peaks at λ_{max} 306, 345 and 378 nm only.

The structure (**4**) for the cyclization products is further proved by examining the nmr spectrum of (**4a**), which showed two broad singlets at δ, 14.38 (1H, s, triazole NH) and δ, 12.29 (1H, s, indole NH). The signals for the aromatic protons appeared as three multiplets around δ, 7.3-8.2 integrating for four protons, consistent with the assigned structure. The mass spectrum of triazolotriazinoindole (**4b**) also showed an intense molecular ion peak at m/z 276, thus confirming the assigned structure. The melting point, analytical and spectral data of compounds (**4**) are given in Table 1.

The triazolotriazinoindoles (**4**) are then subjected to Mannich reaction using formaldehyde and amines such as morpholine, piperidine and 2,4-dichloroaniline. The products of such reactions are assigned structure (**5**). This suggests that aminomethylation occurred at the triazole ring and not at the indole ring. This fact was further verified by the synthesis of triazolotriazinoindole (**10**) (Scheme 2), which was different from (**5b**). The two compounds (**5b**) and (**10**) were distinctly different in colour, mp and uv spectral data. The structure of the Mannich base (**5h**) was further confirmed by examining its mass spectrum, which showed a weak molecular ion peak at m/z 353. The mass spectra of (**5b**) and (**5j**) were also recorded and were in conformity with the assigned structures

The preliminary screening of the new synthetic compounds (**4**) and (**5**) for their antibacterial activity against Staphylococcus aures , Aerobacter aerogenes, Bacillus subtilis and Escherichia coli by disc diffusion method did not show any significant activity even at 50 μg/ml concentrations. A few selected compounds were screened for their antiviral properties. Results of such studies are given in Table 2.



(i) HCHO, NH / EtOH (ii) $NH_2NHCSNH_2$, K_2CO_3 / H_2O

(iii) $N_2H_4 \cdot H_2O$ (iv) CS_2 / KOH, MeOH

Scheme 2

Table 1: Characterization data of 5-mercapto-s-triazolo[3,4-c]-as-triazino[5,6-b]indoles (**4**) and their Mannich bases (**5**)

Compound No.	R	R^1	$-N(R^2)(R^3)$	mp ^0C	yield %	Molecular formula	Analysis, % Found (Calcd) C	H	N	Mass (% abundance) M^+	$M^+ - HCN(R^2)(R^3)$
4a	H	H	-	>350	70	$C_{10}H_6N_6S$	49.10 (49.59	2.65 2.48	34.63 34.71)	242 (100)	-
4b	Cl	H	-	>350	68	$C_{10}H_5N_6ClS$	43.82 (43.39	2.06 1.81	30.65 30.38)	276 (31.8)	-
4c	Me	H	-	>350	65	$C_{11}H_8N_6S$	51.09 (51.56	3.38 3.13	12.35 12.50)		
4d	H	Et	-	345-46	58	$C_{12}H_{10}N_6S$	52.85 (53.33	3.44 3.70	30.97 31.11)		
4e	Cl	Me	-	>350	63	$C_{11}H_7N_6ClS$	45.72 (45.44	2.26 2.41	29.11 28.92)		
5a	H	H	Morpholino	185	52	$C_{15}H_{15}N_7OS$	53.08 (52.79	4.13 4.40	28.5 28.74)		
5b	H	H	Piperidino	235-37	62	$C_{16}H_{17}N_7S$	56.28 (56.64	4.89 5.02	28.76 28.91)	*	242 (23.3)
5c	H	H	2,4-Dichloro-anilino	335-36	65	$C_{17}H_{11}N_7Cl_2S$	49.35 (49.04	2.47 2.64	23.35 23.56)		
5d	Cl	H	Morpholino	255-57	58	$C_{15}H_{14}N_7ClOS$	47.83 (47.94	3.58 3.73	26.34 26.10)		
5e	Cl	H	Piperidino	204	53	$C_{16}H_{16}N_7ClS$	50.98 (51.41	4.11 4.28	26.31 26.24)		
5f	Cl	H	2,4-Dichloro-anilino	278	60	$C_{17}H_{10}N_7Cl_3S$	45.74 (45.28	2.04 2.22	21.57 21.75)		
5g	Me	H	Morpholino	265-66	65	$C_{16}H_{17}N_7OS$	54.53 (54.08	4.61 4.79	27.33 27.61)		
5h	Me	H	Piperidino	277	70	$C_{17}H_{19}N_7S$	58.05 (57.79	5.19 5.38	27.56 27.76)	353 (11.4)	256 (25.0)
5i	Me	H	2,4-Dichloro-anilino	250	55	$C_{18}H_{13}N_7Cl_2S$	49.87 (50.23	2.92 3.02	22.96 22.79)		
5j	Cl	Me	Morpholino	>340	75	$C_{16}H_{16}N_7ClOS$	49.47 (49.29	4.33 4.11	25.38 25.16)	*	290 (50.0)
5k	Cl	Me	2,4-Dichloro-anilino	>340	68	$C_{18}H_{12}N_7Cl_3S$	46.98 (46.50	2.72 2.58	21.23 21.10)		

* Molecular ion peak was not observed. All compounds were recrystallized from dimethylformamide or aqueous dimethylformamide.

Table 2 : Virology data of compounds (**4**) and (**5**)

Activity against herpes simplex virus (HSV) types 1 and 2 in VERO cells and human rhinovirus (HRV) types IA and 39 in W138 cells and cytotoxicity of selected compounds against KB cells.

Toxicity and antiviral activity : minimum inhibitory concentration is in units of μg/ml

Compound No.	Toxicity (VERO)	HSV 1	HSV 2	Toxicity (W138)	HRV 1A	HRV 39	Toxicity (KB)
4a	>50	>50	>50	>50	>50	>50	--
4c	50	>50	>50	>25	>25	25	--
4d	50	>50	>50	>50	>50	>50	--
4e	50	>50	>50	25	>25	>25	--
5b	25	>25	>25	50	>50	>50	--
5c	50	>50	>50	50	>50	>50	--
5f	50	>50	>50	50	>50	>50	--
5g	10	>10	>10	25	>25	>25	>10
5h	50	>50	>50	>50	>50	>50	--
5j	50	>50	>50	25	>25	>25	--
5k	50	>50	>50	25	>25	>25	--
ACV	10	0.2	1	--	--	--	--
WIN	--	--	--	50	10	5	--
5F'URD	--	--	--	--	--	--	0.5

-- = Not tested, ACV =Acyclovir, WIN = Disoxaril, 5F'URD = 5- Fluorouridine

It can be seen from the table that only one compound possessed antiviral property comparable to that of the standard drug, namely, 5-fluorouridine. However, the cytotoxic studies revealed that the synthetic compound was more toxic than the standard drug.

EXPERIMENTAL

All melting points are uncorrected. The ^{1}H nmr spectra in DMSO-d_6 were recorded with a 270 MHz NMR spectrometer. Mass spectra of some selected compounds were recorded on a JEOL JMS-D300 mass spectrometer operating at 70eV. The uv spectra were measured in dimethylformamide using Beckman DU-6 spectrophotometer.

All substituted anilines employed for the preparation of isatins were obtained commercially and were used after distillation. Isatins and N-methylisatins were prepared according to the literature methods.[14-15] 3-Mercapto-as-triazino[5,6-b]indoles were prepared as per the procedure reported by us earlier.[10]

General procedure for the synthesis of 5-mercapto-s-triazolo[3,4-c]-as-triazino[5,6-b]indoles (**4**)

A mixture of triazinoindolylhydrazine (**3**) (0.9 g, 0.0045 mol) in methanol (60 ml), potassium hydroxide (0.1 g, 0.0054 mol) and carbon disulphide (3 ml, 0.0496 mol) was refluxed for 4 h. The reaction mixture was filtered, concentrated and neutralized with acetic acid, whereby, a dark reddish yellow material precipitated. It was filtered, dried and recrystallized from dimethylformamide. The analytical, spectral and physical data of these compounds (**4**) are given in Table 1.

General procedure for the synthesis of 4-aminomethyl-5-mercapto-s-triazolo[3,4-c]-as-triazino[5,6-b]indoles (**5**)

A solution of the appropriate triazolotriazinoindole (**4**) (0.01 mol), formaldehyde (40%, 1.5 ml) and suitably substituted amine (0.01 mol) in dimethylformamide (50 ml) was stirred for 1 h and left over night at room temperature. The solid mass which separated was collected by filtration, washed with ethanol, dried and recrystallized from dimethylformamide. The analytical, spectral and physical data of these Mannich bases (**5**) are given in Table 1.

N-Piperidinomethylisatin (**7**) - This compound was prepared by treating isatin with a mixture of piperidine and formaldehyde in ethanol as per reported procedure;[13] mp. 141^{0}C.

N-Piperidinomethyl-3-mercapto-as-triazino[5,6]indole (**8**) - This compound was prepared by refluxing an equimolar mixture of (**7**) and thiosemicarbazide in the presence of potassium carbonate in water medium according to the method described earlier ;[10] mp. 163-165^{0}C

N-Piperidinomethyl-3-hydrazino-as-triazino[5,6-b]indole (**9**) - This compound was prepared by refluxing (**8**) with hydrazine hydrate according to the reported method ;[10] mp. 268^{0}C. Anal. Calcd for $C_{15}H_{19}N_7$: C, 60.61; H, 6.40; N, 32.99. Found : C, 60.96; H, 6.20; N, 32.67 .

N-Piperidinomethyl-5-mercapto-s-triazolo[3,4-c]-as-triazino[5,6-b]indole (**10**) - A mixture of (**9**), carbon disulphide and potassium hydroxide in methanol (50 ml) was refluxed for 4 h. The reaction mixture on usual workup yielded reddish micro crystals of (**10**); mp. 287-288^{0}C. Anal. Calcd for $C_{16}H_{17}N_7S$: C, 56.64; H, 5.02; N, 28.91. Found : C, 56.08; H, 5.15; N, 28.63 .

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