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Studies on Condensed Triazines as Chemotherapeutic Agents, II[1)]

Synthesis of 1,2,4-Triazino[5,6-*b*]indoles and Related Compounds

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1,2,4-Triazino[5,6-*b*]indoles and their derivatives are prepared by the cyclisation of isatin 3-thiosemicarbazone **(1)** followed by condensation and displacement reactions. Condensations of 3-hydrazino-1,2,4-triazino[5,6-*b*]indole **(4)** with ethoxymethylenemalononitrile, ethyl ethoxymethylenecyanoacetate, acetylacetone and methyl bis(methylmercapto)methylenecyanoacetate yield

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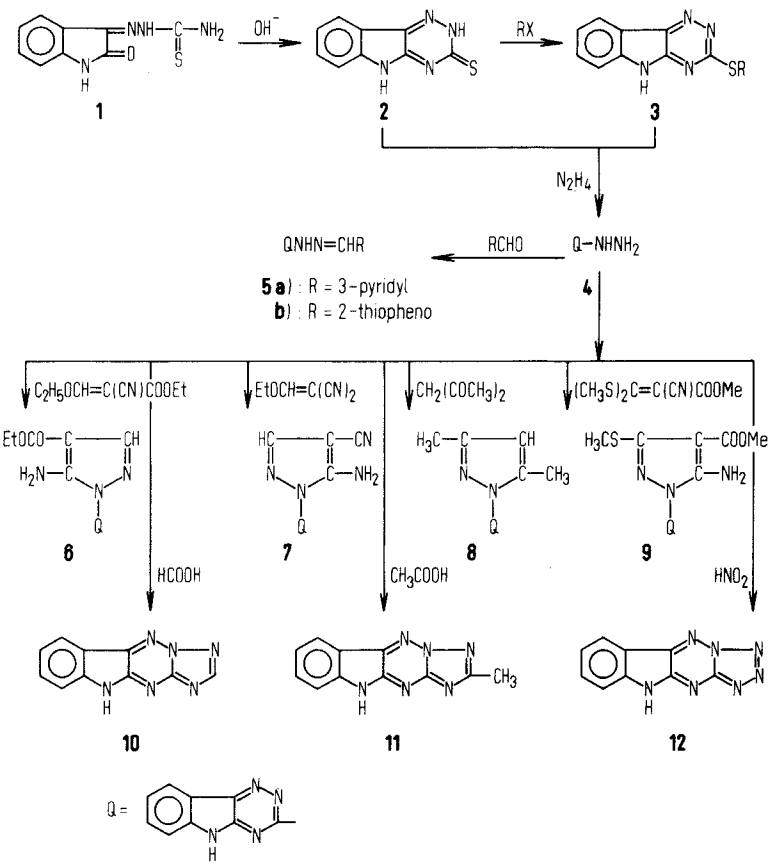
the corresponding 3-pyrazolo-1,2,4-triazino[5,6-*b*]indoles **6–9**. The hydrazine **4** is further transformed into the higher heterocycles **10–12** on reaction with formic, acetic or nitrous acids.

Über kondensierte Triazine als chemotherapeutische Wirkstoffe, 2. Mitt.: Synthese von 1,2,4-Triazino[5,6-b]indol und verwandten Verbindungen

1,2,4-Triazino[5,6-b]indole und ihre Derivate werden durch Cyclisierung von Isatin-3-thiosemicarbazon **(1)** gefolgt von Kondensations- und Umlagerungsreaktionen hergestellt. Die Kondensation von 3-Hydrazino-1,2,4-triazino[5,6-b]indol **(4)** mit Ethoxymethylenmalononitril, Ethoxymethylencyanessigsäureethylester, Acetylaceton und Bis(methylmercapto)-methylencyanessigsäuremethylester ergibt die entsprechenden 3-Pyrazolo-1,2,4-triazino[5,6-b]indole **6–9**. Durch Umsetzung mit Ameisensäure, Essigsäure oder salpetriger Säure kann das Hydrazin **4** in die höheren Heterocyclen **10–12** umgewandelt werden.

The greater recognition of triazine as chemotherapeutic agent led to extensive investigation in the field of condensed triazines especially those fused with a heterocyclic moiety. The versatile therapeutic utility of condensed triazines is described in the previous communication[1]. The pyrimidino [5,6-b]1,2,4-triazine derivatives Toxaflavin and Fervenulin have aroused considerable interest because of their broad spectrum antibacterial[2], antifungal, antiparasitic and anticancer[3] activities. The recognition of triazines as a class of diuretic agents stemmed from the work of *Lipschitz*[4]. The reported antiviral activity of as-triazines[5] and the well documented clinical efficacy of methisazone[6] prompted us to prepare a series of triazino[5,6-b]indoles. The isatin 3-thiosemicarbazone was reported active, mainly against poxvirus[7]. Thus it was pertinent to prepare 1,2,4-triazino[5,6-b]-indoles from the viruscidal intermediate, isatin-3-thiosemicarbazone in the anticipation that the title compounds will exhibit the combined properties.

The key intermediate, isatin-3-thiosemicarbazone **(1)** used for the synthesis of the title compounds, was prepared by the literature procedure[8] and was further cyclised into 1,2,4-triazino[5,6-b]indole-3-thione[9] **(2)** in aqueous potassium carbonate. Alkylation of the thione group in alkaline medium gave the alkylthio derivatives **3**. Reaction of **2** or **3** with hydrazinehydrate (80 %) gave 3-hydrazino-1,2,4-triazino-[5,6-b]indole **(4)** which is a good precursor for the synthesis of higher heterocycles and other derivatives. The condensation of **4** with ethylethoxymethylenecyanoacetate, ethoxymethylenemalononitrile, acetylacetone and dimethylmercaptomethylenemethylcyanoacetate yielded the 3-pyrazolo-1,2,4-triazino[5,6-b]indoles **6–9**. Reaction of **4** with formic and acetic acid gave 1,2,4-triazolo[2,3-b]-1,2,4-triazino[5,6-b]indole **(10)** and 5-methyl-1,2,4-triazolo[2,3-b]1,2,4-triazino[5,6-b]indole **(11)**, while the reaction with nitrous acid afforded 1,2,3,4-tetrazolo[1,5-b]1,2,4-triazino[5,6-b]indole **(12)** in good yield. By condensation of **4** with heterocyclic aldehydes the corresponding hydrazones were obtained. All the compounds were characterised by elemental analyses and spectroscopical studies.



Experimental

All the m.ps. are uncorrected.

1,2,4-Triazino[5,6-b]indole-3-thione[8] **(2)**

A mixture of 0.1 mole isatin, 0.1 mole thiosemicarbazide and 0.15 mole potassium carbonate in 500 ml water was refluxed with stirring for 8 h. On cooling the mixture was filtered and acidified with acetic acid. The precipitate was washed with water and finally crystallised from DMF, m.p. > 300°.

A General Procedure for the Synthesis of 3-Alkylthio-1,2,4-triazino[5,6-b]indoles **(3)**

To a solution of **2** in aqueous sodium hydroxide (4 %), alkyl halide was added during 2–5 min with stirring. The mixture was stirred for 1 h and the precipitate was washed with water. All the sulphides thus prepared (Table 1) were crystallised from a DMF-water mixture.

Table 1: *3-Alkylthioindoles* **3**

No. 3+	R	M+	Yield %	M.P. °C	Molecular formula	U.V. (CH_3OH + HCl) λ_{max} (log ϵ) nm
a	Ethyl	230	60	118[r]	$C_{11}H_{10}N_4S$	344 (3.65), 283 (4.61)
b	n-Propyl	244	65	272	$C_{12}H_{12}N_4S$	343 (3.66), 282 (4.68), 231 (3.87)
c	Allyl	242	45	280	$C_{12}H_{10}N_4S$	– –
d	Benzyl	292	62	280	$C_{16}H_{12}N_4S$	344 (3.65), 283 (4.67)
e	p-Bromobenzyl	371	58	270	$C_{16}H_{11}BrN_4S$	– –

+ All the compounds were analysed for C, H & N satisfactorily and crystallised from DMF.
r = Reported[8] m.p. 304°.

3-Hydrazino-1,2,4-triazino-[5,6-b]indole **(4)**

A mixture of 0.01 mole 3-methylthio-1,2,4-triazino[5,6-b]indole or **2** and 10 ml hydrazine hydrate (98 %) was refluxed for 4 h. On cooling a light yellow solid separated which was washed with water, methanol subsequently and crystallised from DMF; yield 47.4 %, m.p. 310–12° (Rep.[8] > 300°).
MS: M^+, 200; 169($-NHNH_2$); IR: 3350, 3260, 3170 (ν NH); 1620 cm^{-1} (δ NH).

3-(Pyridine-3-aldehyde)hydrazono-1,2,4-triazino[5,6-b]indole **(5a)**

An equimolar mixture of **4** and pyridine-3-aldehyde in dimethylformamide was refluxed for 1 h. During this period a yellow solid separated from the solution which was crystallised from DMF. The yield was quantitative, m.p. > 300°.
$C_{15}H_{11}N_7$ (289) Calc.: C 62.3 H 3.8 N 33.9 Found: C 62.1 H 3.7 N 33.7.

3-(Thiophene-2-aldehyde)hydrazone-1,2,4-triazino[5,6-b]indole **(5b)**

5b was prepared from 0.2 g thiophene-2-aldehyde and 0.3 g **4** following the above procedure in quantitative yield, m.p. > 300°.
$C_{14}H_{10}N_6S$ (294) Calc.: C 57.1 H 3.4 N 28.6 Found: C 57.3 H 3.5 N 28.3. MS: M^+ 294; 211($-C_4H_3S$); 185(−CN); 169($-NH_2$); 143 (−CN).

3-(4'-Carbethoxy-5'-aminopyrazol-1'yl)-1,2,4-triazino[5,6-b]indole **(6)**

A mixture of 0.2 g **4** and 0.2 g ethylethoxymethylenecyanoacetate in 8 ml ethanol was refluxed for 2 h. During this period a yellow solid separated which was crystallised from DMF, yield 0.25 g, m.p. > 300°.
$C_{15}H_{13}N_7O_2$ (323) Calc.: C 55.7 H 4.05 N 30.3 Found: C 55.5 H 3.9 N 30.5 MS: M^+ 323; 277(−EtOH); 251(−CN); 169(-Pyrazole ring $C_6H_8N_3O_2$).
U.V. (MeOH + HCl, pH O) λ max (log ε): 275 (4.23); 229 nm (2.94). IR(KBr): 1700 (ν CO); 3426 3318 cm^{-1} (ν NH).

3-(4'-Cyano-5'-aminopyrazol-1'yl)-1,2,4-triazino[5,6-b]indole **(7)**

A solution of 0.2 g ethoxymethylenemalononitrile in 8 ml ethanol was mixed with 0.2 g **4**. The mixture

was refluxed for 2 h, cooled and filtered. The crude product was crystallised from DMF; yield 0.3 g, m.p. > 300°.
$C_{13}H_8N_8$ (276) Calc.: C 56.5 H 2.9 N 40.6 Found: C 56.6 H 3.1 N 40.7. MS: M^+ 276; 248(N_2); 169 (pyrazole ring, $C_4H_3N_3$). IR(KBr): 3400 (νNH) 2220 cm^{-1} (νCN).

3-(3',5'-Dimethylpyrazol-1'yl)-1,2,4-triazino[5,6-b]indole **(8)**

A mixture of 0.5 ml acetylacetone and 0.2 g **4** in 15 ml ethanol containing a few drops of acetic acid was refluxed for 2 h. The solution was cooled and a light brown solid separated. The crude product was crystallised from DMF/water; yield 0.2 g, m.p. > 300°.
$C_{14}H_{12}N_6$ (264) Calc.: C 63.6 H 4.58 N 31.8 Found: C 63.7 H 4.4 N 32.1. MS: M^+ 264; 249(−CH_3); 223(−CN); 208(−CH_3); 169(pyrazole ring, $C_5H_7N_2$).

3-(3'-Methylmercapto-4'-carbethoxy-5'-aminopyrazol-1'yl)-1,2,4-triazino[5,6-b]indole **(9)**

0.22 g Dimethylmercaptomethylenemethylcyanoacetate and 0.2 g **4** were refluxed in 10 ml ethanol for 3 h. During this period an orange precipitate separated which was washed with ethanol and crystallised from DMF. The yield was quantitative, m.p. 273°.
$C_{15}H_{13}N_7O_2S$ (355) Calc.: C 50.7 H 3.7 N 27.6 Found: C 50.5 H 3.8 N 27.7. MS: M^+ 355; 323(MeOH); 296(−COOMe); 276(323-SCH_3).

sym-Triazolo[4,3-b]-1,2,4-triazino[5,6-b]indole **(10)**

A solution of 0.2 g **4** in 5 ml formic acid was refluxed for 4 h. Excess of solvent was removed. The crude product was crystallised from DMF; yield 0.14 g, m.p. > 300°.
$C_{10}H_6N_6$ (210) Calc.: C 57.1 H 2.9 N 40.0 Found: C 57.2 H 3.1 N 40.2. MS: M^+ 210; 182(−N_2); 155(−HCN); 128(−HCN).

3'-Methyl-1',2',4'-triazolo[4,3-b]-1,2,4-triazino[5,6-b]indole **(11)**

11 was prepared by refluxing 0.2 g **4** in 5 ml acetic acid. The product was crystallised from DMF; yield 0.15 g, m.p. > 300°.
$C_{11}H_8N_6$ (224) Calc.: C 58.9 H 3.6 N 37.5 Found: C 58.8 H 3.7 N 37.6. MS: M^+ 224; 155(CH_3CN,N_2); 128(HCN); 102(−CN).

Tetrazolo[1,5-b]1,2,4-triazino[5,6-b]indole **(12)**

To a solution of 0.2 g **4** in 8 ml 50 % acetic acid, 0.2 g sodium nitrite in aqueous solution was added gradually and the resulting mixture was stirred for 2 h at room temp. The crude orange precipitate was crystallised from DMF; yield 0.13 g, m.p. > 300°.
$C_9H_5N_7$ (211) Calc.: C 51.2 H 2.4 N 46.4 Found: C 51.3 H 2.5 N 46.2. MS: M^+ 211; 185(−CN); 157(−N_2); 129(N_2).

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Trennung des Aloins in Diastereomere und deren Charakterisierung

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Aloin wurde durch Hochdruckflüssigkeitschromatographie in Aloin A und Aloin B getrennt. Aloin A läßt sich aus Aloin auch durch mehrfache Kristallisation aus Methanol rein gewinnen. Die Aloine unterscheiden sich insbesondere durch ihre optischen Drehwerte und circulardichroitischen Effekte. Wie die Partialsynthese aus Anthronen und α-Bromacetoglucose zeigte, ist in beiden Aloinen der Glucosylrest β-ständig mit dem Aloeemodinanthron verbunden; C-1′ des Glucosylrestes ist *R*-konfiguriert. Aloin A und B unterscheiden sich durch die Konfiguration von C-10 des Anthronrestes. Die Auswertung der ^{1}H-NMR-Spektren macht für Aloin A die 10 *R*, 1′*R*-Konfiguration wahrscheinlich, für das chemisch labilere Aloin B die 10 *S*,1′*R*-Konfiguration.

Resolution of Aloin into Diastereomers and Their Characterization

Aloin was separated into aloin A and aloin B by HPLC. Aloin A may also be obtained by crystallization of aloin from methanol. The two aloins differ mostly in optical rotation and circular dichroism. The synthesis from anthrones and acetobromoglucose shows that in both aloins the anthrone is in the β-position of the D-glucose. C-1′ of the D-glucose has the *R*-configuration. Aloin A and aloin B have different configurations at C-10 of the anthrone part. ^{1}H-NMR spectra show that aloin A is the 10 *R*,1′*R*- compound, whereas the chemically less stable aloin B is the 10*S*, 1′-*R*-compound.

Handelsübliche Aloine haben sehr unterschiedliche physikalische Eigenschaften. Wie Dünnschichtchromatogramme zeigen, sind manche Produkte bloße Aloeextrakte, andere, die im DC nur einen Fleck geben, schwanken recht stark im Schmelzpunkt und Drehwert,

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