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Synthesis and Structure of Purine Derivatives as Antitumor Effects

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Abstract□The nucleophilic substitution reaction of 6-chloro purines (I) with malononitrile and ethyl cyanoacetate is carried out in DMSO and in the presence of an alkali. The possible tautomeric -ylidene form for the products is considered and discussed in view of IR, UV, NMR and mass spectral determinations. The derivatives were tested for their antitumor activities.

Keywords□6-Chloro-9-aryl-9H-purines, purinylidenes, antitumor activity.

Because many purines have been claimed in the last decades to possess numerous useful antiviral[1] and antitumer[2, 3] agents, we have developed new syntheses of purine derivatives of antitumor activity.[4, 5] The aim of this work was to synthesize purine derivatives and test their potential antitumor activities.

The aromatic nucleophilic substitution reaction of acylic active methylene compounds with heterocyclic systems possessing replaceable halogen has been previously recieved some attention.[6] Also, some 6-cyanomethylene purines were prepared by hetero-arylation of the sodium solt of an active methylene containing cyano groups with 6-methylsulfonated purines; but no studies on the tautomeric possibilities for the resulting products, apparently, were considered.[7]

As part of our study on the synthesis of 6-C-substituted purine derivatives of potential useful biological activity[8] we wish to report, in the present article, our result on the nucleophilic substitution reaction of malononitrile and ethyl cyanoacetate with 6-chloro-9-aryl-9H-purines as well as structural tautomeric possibilities for the obtained products.

Thus, when 6-chloro-9-aryl-9H-purines of type **I** were left to react, for a short time, with malononitrile or ethyl cyanoacetate in dimethyl sulfoxide (DMSO) solution and in the presence of an alkali; replacement of the chloro substituent with the dicyanomethanide or the cyano (ethoxycarbonyl) methanide anion occurred and the corresponding dinitriles (**IIa-f**) or the nitrile esters (**IIg-1**) were smoothly obtained in good yield.

However, products **IIa** and **f** have been previously reported[9] and given the structure of type (**II**); but careful inspection for i.r., u.v. and n.m.r. spectra of most of the products, obtained in the present investigation, proved their existance in the -ylidene form of type (**IIA**) in both solid or liquid phases.

The infrared spectra (KBr-disc) of the dinitriles (**IIa-f**) showed split nitrile absorption at 2230 and 2200 cm^{-1} beside anothe absorption around 3200 cm^{-1} region which could be ascribed to NH vibration. Also, the ester carbonyl absorption in the spectra of the nitrile esters (**IIg-1**) does not occur in the normal position *i.e.* in the 1770-1770 cm^{-1} region; instead, there is a strong absorption near 1645 cm^{-1}. Assuming that this band is due to the carbonyl absorption; this abnormal frequency shift can be rationalized in terms of the contribution of a structure such as (**IIB**) to the ground state of the molecule[10]. The frequency shift in this case is probably bacause of intramolecular hydrogen bonding effects.

The ultraviolet absorption spectra (ethanol) of products (**IIc, f, g** and **k**) are found to exhibit absorption maxima at 335-343 nm region. In com-

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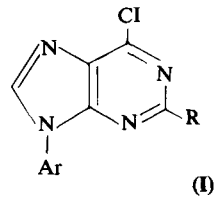
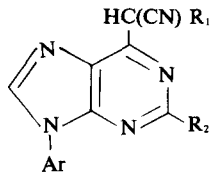
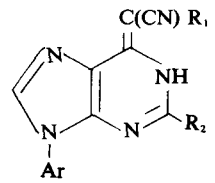


(I)

I	Ar	R	I	Ar	R
a	C_6H_5	H	d	*p*-ClC_6H_4	H
b	*p*-$CH_3C_6H_4$	H	e	*p*-$CH_3C_6H_4$	CH_3
c	*p*-$CH_3OC_6H_4$	H	f	*p*-$CH_3OC_6H_4$	CH_3

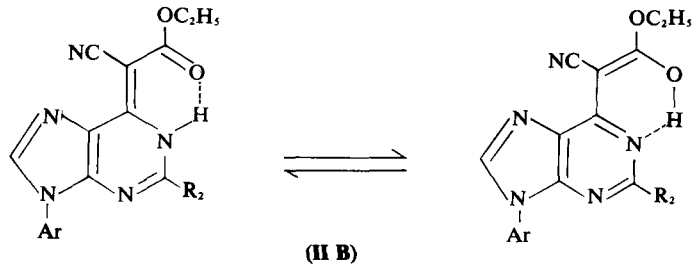


(II)



(II A)

II	Ar	R_1	R_2	II	Ar	R_1	R_2
a	C_6H_5	CN	H	g	C_6H_5	$CO_2C_2H_5$	H
b	*p*-$CH_3C_6H_4$	CN	H	h	*p*-$CH_3C_6H_4$	$CO_2C_2H_5$	H
c	*p*-$CH_3OC_6H_4$	CN	H	i	*p*-$CH_3OC_6H_4$	$CO_2C_2H_5$	H
d	*p*-ClC_6H_4	CN	H	j	*p*-ClC_6H_4	$CO_2C_2H_5$	H
e	*p*-$CH_3C_6H_4$	CN	CH_3	k	*p*-$CH_3C_6H_4$	$CO_2C_2H_5$	CH_3
f	*p*-$CH_3OC_6H_4$	CN	CH_3	l	*p*-$CH_3OC_6H_4$	$CO_2C_2H_5$	CH_3



(II B)

parison, the spectra of 6-chloro-9-substituted -9H-purines were previously reported[11] to show no absorption bands beyond 270 nm. Observation of such long wave-length bands in the spectra of products (**II**) could be considered as an indication for the presence of an unsaturated moiety on the purine nucleus. Noteworthy to notice also that the spectra of **IIf** and **k** are not changed, considerably, when repeated in chloroform solution. This could suggest the predominance of the products under investigation in the -ylidene form (**IIA**) in both hydroxylic and non-hydroxylic solvents.

The ^1H NMR spectra of products **IIb** and **j** included signals representing C-2 and C-8 of the purine ring,[12] while those of (**IIe, f, k**) and (**I**) showed C-8 proton signals beside the signals due to C-2 methyl protons. All the investigated spectra cleared absence of the expected CH on C-6 of the purine ring) proton signals which is found in structure of type (**II**). Also, careful examination for

spectra of products (**IIe**) and **j** showed signals at δ 8.7 and 8.55 ppm, respectively, which could be ascribed to NH proton found in the -ylidene structure (**IIA**)

Moreover, the mass spectra of products (**IIb**) and (**c**) observed M274 and 290.3,respectively.

So, aromatic substitution reaction of the active methylenes: malononitrile and ethyl cyanoacetate with 6-chloro-9-aryl-9H-purines is presented, now, and the -ylidene form of type (**IIA**) must be considered in assigning structure to the obtained products rather than the structure of type (**II**).

The newly prepared purine derivatives were tested for antitumor activities.

From these results we can conclude that i) Introduction of 9-(4 '-chlorophenyl) derivatives improved the antitumor activity of purine derivatives with increasing the mean survival time to 17.5 days in compare to 13.7 days in the untreated control group. 9-(4 '-chlorophenyl)-6-dinitrile purine (**IIb**) showed better effect than 9-(4 '-chlorophenyl)-6-nitrile ethyl ester purine (**IIj**) with mean survival time equal 17.5 and 16.7 days, respectively. ii) 9-(4 '-Methoxyl; 4 '-methylphenyl)-6-dinitrile purine (**II c,b**) and 9-(4 '-methoxyphenyl)-6-nitrile ethylester purine (**IIi**) slight significant against tumor cells but

Table I: The anticancer activity of compound II

Compd.	Dose mg/kg.	No. of Doses	Mean Survival Time(days) ± S.D.	T/C
II b	100	3	14.9 ± 10.3	1.08
c	100	3	16.2 ± 10.8	1.18
d	100	3	17.5 ± 11.2	1.28
e	100	2	12.0 ± 12.7	0.88
f	100	3	12.9 ± 11.3	0.94
h	100	2	13.4 ± 11.5	0.98
i	100	3	14.6 ± 13.5	1.07
j	100	2	16.6 ± 12.4	1.22
k	100	3	12.3 ± 11.7	0.89
l	100	2	11.4 ± 11.5	0.83
Control	-	-	13.7 ± 2.5	1.0

Table II: Characterization of the synthesized purinylidenes(II)

Compound	Mp/ °C (solvent)	Yield %	Formula (M.W.)	Elemental analysis					
				Calcd.			Found		
				C%	H%	N%	C%	H%	N%
II b	300 (DMF)	73	$C_{15}H_{10}N_6$ (274)	65.69	3.65	30.65	65.47	3.55	31.01
c	310-11 (DMF)	72	$C_{15}H_{10}N_6O$ (290)	62.06	3.45	28.96	61.73	3.66	28.62
d	320-22 (DMF)	85	$C_{14}H_7ClN_6$ (294.5)	57.04	2.37	28.52	57.12	2.41	28.38
e	310-302 (n-butanol)	69	$C_{16}H_{12}N_6$ (288)	66.67	4.17	29.17	66.50	4.50	29.40
f	300 (n-butanol)	72	$C_{16}H_{12}N_6O$ (304)	63.16	3.95	27.63	63.12	4.01	27.63
h	328-30 (DMF)	81	$C_{17}H_{15}N_5O_2$ (321)	63.55	4.67	21.81	63.61	4.55	22.03
i	300 (DMSO)	68	$C_{17}H_{15}N_5O_3$ (337)	60.53	4.45	20.77	60.29	4.37	20.37
j	300 (DMSO)	82	$C_{16}H_{12}ClN_5O_2$ (341.5)	56.22	3.51	20.51	55.99	3.33	20.83
k	242-44 (n-butanol)	75	$C_{18}H_{17}N_5O_2$ (335)	64.48	5.07	20.89	64.60	4.65	20.49
l	250-52 (n-butanol)	77	$C_{18}H_{17}N_5O_3$ (351)	61.54	4.84	19.94	61.89	4.78	20.00

Table III: Spectral properties of the synthesized purinylidenes (II)

Comp.	IR (KBr) cm^{-1}			UV(ethanol)	^1H NMR ($DMSO_{d6}$)	MS m/z
	NH	C=N	CO	max (t)	(δvalue from TMS)	(%intensity)
IIb	3215	2220,			2.40(s, 3H, CH_3); 7.20-	274
					7.50(m, 4H, C_6H_4); 8.20	(100
		2200			(s, 1H, C-8), 8.55(s, 1H, C-2)	
c	3220	2225,		336(34220)		290.31
		2200		239(14500)		(57.90)
d	3200	2235,				
		2210				
e	3250	2220,			2.60(m, 6H, $2CH_3$); 7.30-	
		2200			7.80(m. 4H, C_6H_4); 8.50	
					(s. 1H, C-8); 8.7(s, 1H, NH).	
f	3250	2225,		335(23900),	2.60 (s, 3H, CH_3); 3.90 (s,	
		2205		235(12160),	3H, OCH_3); 7.1-7.8(m, 4H,	
				202(24250)	C_6H_4; 8.55(s, 1H, C-8).	
g		2180	1640	342(35042),		
				229(27660).		
h		2180	1645			
i		2180	1645			
j		2180	1645		1.25(t, 3H, CH_3); 4.10(q, 2H,	
					CH_2); 7.50-8.00 (m, 4H, C_6H_4);	
					8.23(s, 1H, C-8); 8.40 (s, 1H,	
					C-2); 8.55(s, 1H, NH).	
k		2210	1640	343(39500),	1.30(t, 3H, CH_3); 2.60(m, 6H,	
				244(15530),	$2CH_3$); 4.30(q, 2H, CH_2); 7.35-	
				202(35500)	7.85 (m, 4H, C_6H_4); 8.60 (d, 1H,	
					C-8).	
l		2220	1645		1.30(t, 3H, CH_3); 2.60(s, 3H, CH_3);	
					3.90(s, 3H, OCH_3); 4.30(q, 2H,	
					CH_2); 7.00-7.80(m, 4H, C_6H_4);	
					8.60(s, 1H, C-8).	

IIc showed better significant than **IIb** and **IIi**, with mean survival time equal 16.2 and 14.9, 14.6 days respectively. iii) On the other hand, 9-(4'-methyl)-2H-6-nitrile ethylester and 9-(4'-methoxy)-2-methyl -6-dinitrile purine we showed increased the toxicity than chloro compounds. This has been detected by increasing the montality of the treated animals. 9-(4'-Methyl)-6-nitrile ethyl ester, 2H-purine (**IIh**) showed better effect than 9-(4'-methoxy)-6-dinitrile-2-methyl purine (**IIf**), 9-(4'-methyl)-6-nitrile ethylester-2-methyl purine(**IIe**) and 9-(4'-methoxy)-6-nitrile ethylester-2-methyl purine (**III**) with mean survival time equal 13.4, 12.9, 12.3, 12.0 and 11.4 days, respectively.

EXPERIMENTAL

Melting points are uncorrected. Microanalysis were carried out by the microanalytical Dept., NRC, Cairo, Egypt. IR spectra were recorded with a Perkin-Elmer 580 spectrophotometer, UV spectra with a Beckman part N0 580216 spectrophotometer, and mass spectra with a Varian MAT 311A and a Varian MAT CH 7A mass spectrometer. NMR spectra are measured at 90MHz in $(CD_3)_2SO$ with chemical shifts in from internal Me_4 Si.

9-Aryl-6-chloro-9H-purines (**I**) wer prepared from the corresponding hypoxanthines and $POCl_3$ according to the previously reported method.[13]

Preparation of the purinylidenes (II)

A mixture of (**I**, 0.01 mol), malononitrile or ethyl cyanoacetate (0.012 mol) and potassium hydroxide (2.40 gm in 3 ml water) in DMSO (30 ml) was heated on a water-bath, while stirring, for one hr. After cooling, the reaction mixture was diluted with cold water (10 ml) and then acidified with cold dilute acetic acid solution. The precipitate obtained was filtered off, washed with water, dried, then crystallized from the suitable solvent to give the puprinylidenes (**II**). (cf. Tables II and III)

Antitumor Activity

Evaluation of the antitumor activity of derivatives: Female Swiss albino mice weighing 18-20gm were used in this study from the colony of the Cancer Institute, Cairo University. Animals were maintained on standard diet and a free supply of water.

A line of Erich ascites carcinoma was used. The tumor was kindly supplied by Dr. G. KLEIN, Amesterdam and was maintained by serial intraperitoneal transplantation of 2.5×10^6 tumor cells from a donor mice (7 days old) and the drug in the appropriate dose was administered after 24 hr. of tumor inoculation for a total 5 injections every other day. The mice were observed for a period of two months or to the death of the last animal. The mean servival time of the treated animals were compared with that of untreated animals (Table I).

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