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SYNTHESIS OF SOME FUSED PYRIMIDINE DERIVATIVES WITH EXPECTED BIOLOGICAL ACTIVITY

A.H. Abd El-Aleem, M. Zahran and H. Marouf

Chemistry Department, Faculty of Science, Menoufia University, Shebin El-Koam, Egypt.

ABSTRACT

Pyrazolo[3,4-d]pyrimidine ***1*** *condensed with acetyl-acetone, ethylacetoacetate in methanol, ethylacetoacetate in Acetic anhydrid, phenylisocyanate and ethoxy-methylenecyanoacetate under several reactions conditions afforded the corresponding pyrazolo[3,4-d]pyrimidine derivatives* ***4, 5, 6, 7 and 8,*** *respectively. On the other hand* ***1*** *reacted via diazotization reaction with 1,3-dimethyl-barbituric acid, thiobarbituric acid and pyrazolone to give the corresponding hydrazone derivatives* ***9, 10 and 11*** *respectively.*

Also 6-chloro-5-cyano-1,3-dimethyluracil ***3*** *reacted with methyl and phenyl-hydrazine, semicarbazide, thiosemi-carbazide, malononitrile and hydroxylamine affording prazolo[3,4-d]pyrimidine derivatives* ***12a,b, 13, 14, 15*** *and* ***16,*** *respectively.*

INTRODUCTION

Several uracil derivatives have been recently developed. Azido-thymidine (AZT)[1] and cyanothymidine (CNT)[2] have been applied successfully as reverse transcriptase inhibitors[3] in AIDS treatment, in various pharmacological activities[4], antibacterial and anticonvulsive

activities.[5] As an extension of the previous investigations[6-8], special attention was drawn to synthesize some new pyrimidine derivatives.

DISCUSSION

The starting material[9] **1** reacted with acetylacetone[10], ethylaceto-acetate in methanol, ethylacetoacetate in acetic anhydride, phenyliso-cyanate[11] and ethoxy-methylenecyanoacetate under different conditions afforded the corresponding pyrazolodipyrimidine derivatives **4, 5, 6, 7** and **8,** respectively.

Infrared spectrum of **4** revealed $\gamma_{2\ C=O}$ at 1700, 1655 cm^{-1} and $\gamma_{C=N}$ at 1620 cm^{-1}. ^{1}H-NMR spectrum of **4** showed δ = 2.6 (s, 3H, CH_3), 2.75 (s, 3H, CH_3) and 3.33 (s, 3H, CH_3) and 3.5 (s, 3H, CH_3). The mass spectrum of **4** showed a molecular ion peak at (m/z, 100%, 259).

Infrared spectrum of **5** showed $\gamma_{3\ C=O}$ at 1695 , 1680 , 1630 cm^{-1} and $\gamma_{C=N}$ at 1595 cm^{-1}. ^{1}H-NMR spectrum of **5** showed δ = 2.3 (s, 3H, CH_3 of pyrimidine ring), 3.25 (s, 3H, CH_3), 3.45 (s, 3H, CH_3, uracil ring), 5.9 (s, 2H, CH_2-). The mass spectrum **5** shows a molecular ion peak at (m/z, 100%, 261).

Infrared spectrum of **6** showed $\gamma_{3\ C=O}$ at 1680 , 1650 , 1630 cm^{-1} and γ_{NH} at 3240 cm^{-1}. ^{1}H-NMR spectrum of **6** showed δ = 2.2 (s, 3H, CH_3), 3.2 (s, 3H, CH_3), 3.35 (s, 3H, CH_3), 10.6 (s, 1H, NH) and 13.1 (s, 1H, NH of pyrazole). The mass spectrum of compound **6** showed molecular ion peak at (m/z, 31%, 237) and base peak at 195.

Infrared spectrum of **7** revealed γ_{NH} at 3300 cm^{-1} and $\gamma_{3C=O}$ at 1710, 1685 and 1655 cm^{-1}, respectively. ^{1}H-NMR spectrum of **7** showed δ = 3.15 (s, 3H, CH_3), 3.35 (s, 3H, CH_3), 7.1-7.7 (m, 5H, arom.) and 9.77 (s, 1H, NH of pyrazole ring). The mass spectrum of **7** showed the base peak at (m/z, 100%, 195).

Infrared spectrum of **8** showed γ_{NH} at 3200 cm^{-1}, $\gamma_{C\equiv N}$ at 2200 cm^{-1} and $\gamma_{3C=O}$ at 1700, 1680 and 1650 cm^{-1} respectively. The mass spectrum of **8** showed molecular ion peak at (m/z, 100%, 318).

The diazotization of **1** followed by condensation with active methylene derivatives, namely dimethylbarbituric acid[12], thiobarbituric acid and pyrazolone gave the corresponding hydrazone derivatives **9**, **10** and **11**, respectively.

Infrared spectrum of **9** showed γ_{OH} at 3290 cm^{-1}, γ_{NH} at 3120 cm^{-1}, $\gamma_{4C=O}$ at 1700, 1670, 1630 and 1620 cm^{-1}, respectively. The mass spectrum of **9** showed molecular ion peak at (m/z, 100%, 362).

Infrared spectrum of **10** showed γ_{OH} at 3325 cm^{-1}, γ_{NH} at 3150 cm^{-1} and $\gamma_{C=S}$ at 1290 cm^{-1} and $\gamma_{3C=O}$ at 1700, 1660 and 1640 cm^{-1}. ^{1}H-NMR spectrum of **10** showed δ = 3.1 (s, 3H, CH_3), 3.4 (s, 3H, CH_3, pyrimidine ring). The mass spectrum of **10** showed molecular ion peak at (m/z, 100%, 350).

Infrared spectrum of **11** showed γ_{OH} at 3200 cm^{-1}, γ_{NH} at 3120 cm^{-1}, $\gamma_{2C=O}$ at 1700 and 1660 cm^{-1}. ^{1}H-NMR spectrum of **11** showed δ = 3.21 (s, 3H, CH_3), 3.35 (s, 3H, CH_3), 3.52 (s, 3H, CH_3) and 7.75-7.90

(m, 5H, arom.). The mass spectrum of **11** showed molecular ion peak at (m/z, 100%, 381).

6-Chloro-5-cyano-1,3-dimethyl uracil **3** reacted with methyl and phenylhydrazine, semicarbazide, thiosemicarbazide[12], malononitrile and hydroxylamine[13] affording pyrazolo[3,4-d]pyrimidine derivatives **12a,b**, **13**, **14**, **15** and **16,** respectively.

Infrared spectra of the above compounds showed $\gamma_{C=O}$ at 1700-1630 cm^{-1} , γ_{NH_2} at 3480-3350 cm^{-1}, 3360-3300 cm^{-1}, γ_{OH} at 3220 cm^{-1}, $\gamma_{C=S}$ at 1280 cm^{-1}, $\gamma_{C\equiv N}$ at 2160 cm^{-1} and γ_{NH} at 3320 cm^{-1}. The mass spectrum of **12a** showed a molecular ion peak at (m/z, 73%, 209) and base peak at m/z 81. ^{1}H-NMR spectrum of **12b** showed δ = 3.15 (s, 3H, CH_3, uracil ring), 3.30 (s, 3H, CH_3, uracil ring), 6.6 (s, 2H, NH_2) and 7.55-7.60 (m, 5H, aromatic protons). The mass spectrum of **12b** showed a molecular ion peak at (m/z, 100%, 271).

^{1}H-NMR spectrum of **13** showed δ = 3.15 (s, 3H, CH_3), 3.27 (s, 3H, CH_3), 7.47 (s, 2H, NH_2, amide NH_2), and 7.8 (s, 2H, NH_2 pyrazole). The mass spectrum of **13** showed a molecular ion peak at (m/z, 21%, 238) and a base peak at m/z 195.

^{1}H-NMR spectrum of **14** showed δ = 3.15 (s, 3H, CH_3), 3.30 (s, 3H, CH_3), 8.65 (s, 2H, NH_2, thioamide) and 9.15 (s, 2H, NH_2 pyrazole ring). The mass spectrum of **14** showed a molecular ion peak at (m/z, 31%, 254) and a base peak at m/z 195.

Malononitrile did not react with **3**, only hydrolysis took place to give compound **15**. ^{1}H-NMR spectrum of **15** showed δ = 3.1 (s, 3H, CH_3)

and 3.4 (s, 3H, CH_3). The mass spectrum of **15** showed a molecular ion peak at (m/z, 95%, 181) and a base peak at m/z 150.

^{1}H-NMR spectrum of **16** showed δ = 3.10 (s, 3H, CH_3), 3.20 (s, 3H, CH_3) and 8.4 (s, 2H, NH_2). The mass spectrum of **16** showed a molecular ion peak at (m/z, 100%, 196).

Antimicrobial Activity

The tested organisms are four Gram-positive (Bacillus subtilis (B.s.) and Saccaromyces servicia (S.s)) and two Gram-negative (E. coli and Pseudomonas gladioli (P.g)) bacteria. The results obtained revealed that the tested compounds **4**, **5** and **6** inhibit the growth of Gram-negative strains in low concentrations. On the other hand, with respect to the Gram-positive strains, it has been found that compounds **4**, **12a,b** and **13** inhibit their growth in high concentrations but less effective in low conentrations.

EXPERIMENTAL PROCEDURES

All melting points are uncorrected and were taken in a Gallenkamp electric melting point apparatus. Infrared spectra were performed on a Perkin-Elmer IR-spectrophotometer 598 (4000-200 cm^{-1}) using KBr wafer technique. Microanalyses were carried out by Microanalytical Unit, Cairo University. ^{1}H-NMR spectra were obtained in DMSO by Varian EM-390 (90 MHz) spectrometer.

Synthesis of 1,3,6,8-tetramethylpyrazolo [3,4-d : 2,3-a] di-pyrimidine-2,4-(1H, 3H)-dione 4:

A mixture of 0.39 g (2 m mol) of **1** and acetylacetone 0.205 ml (2 m mol) in absolute ethanol was refluxed for 2.5 h. The resulting precipitate was collected by filtration and recrystallized from methanol to give 0.29 g (55.9%) m.p. 280-282°C.

Analysis:	$C_{12}H_{13}N_5O_2$	Required:	C, 55.59; H, 5.01; N, 27.02
	(259)	Found:	C, 56.5; H, 4.75; N, 27.3

Synthesis of 7-hydro-1,3,6-trimethylpyrazolo [3,4-d : 2,3-a] di-pyrimidine-2,4,8-(1H, 3H)-trione 5:

A mixture of 0.39 g (2 m mol) of **1** and ethylacetoacetate 0.212 ml (1.7 m mol) in absolute ethanol was refluxed for 10 h. The resulting precipitate was collected by filtration and recrystallized from methanol / chloroform mixture which yielded 0.31 g (53.63%) m.p. >300°C.

Analysis:	$C_{11}H_{11}N_5O_3$	Required:	C, 50.57; H, 4.21; N, 26.8
	(261)	Found:	C, 49.6; H, 4.4; N, 26.5

Synthesis of 3-N-acetyl-5,7-dimethylpyrazolo [3,4-d] pyrimidine-4,6-(5H, 7H)-dione 6:

A mixture of 0.39 g (2 m mol) of **1** and ethylacetoacetate 0.212 ml (1.7 m mol) in acetic anhydride was refluxed for 6 h. The resulting precipitate was collected by filtration and recrystallized from acetone which yielded 0.43 g (54.4%) m.p. 205-207°C.

Analysis:	$C_9H_{11}N_5O_3$	Required:	C, 45.56; H, 4.64; N, 29.53
	(237)	Found:	C, 45.8; H, 4.4; N, 29.2

Synthesis of 5,7-dimethyl-1'-phenylcarbamide-3,3'-pyrazolo [3,4-d] pyrimidine-4,6-(5H, 7H)-dione 7:

A mixture of 0.65 g (3.3 m mol) of **1** and 0.36 ml (3.3 m mol) of phenylisocyanate in dry benzene was refluxed for 17 h. The formed product was filtered and recrystallized from acetone to yield 0.8 g (77%) m.p. 240-241°C.

Analysis:	$C_{14}H_{14}N_6O_3$	Required:	C, 53.50; H, 4.45; N, 26.76
	(314)	Found:	C, 53.20; H, 4.20; N, 26.4

Synthesis of 3-N-methylenecyanoacetate-5,7-dimethylpyrazolo [3,4-d] pyrimidine -4,6-(5H, 7H)-dione 8:

To a mixture of 0.39 g (2 m mol) of **1** and 0.33 g (2 m mol) of ethoxy-methylenecyano acetate in ethanol was added 10 ml ethanolic solution containing (2 m mol) of diethylaminopyridine. The reaction mixture was refluxed for 6 h. The resulting precipitate was collected by filtration and recrystallied from DMF to give 0.29 g (53.7%) m.p. 270-272°C.

Analysis:	$C_{13}H_{14}N_6O_4$	Required:	C, 49.05; H, 4.40; N, 26.40
	(318)	Found:	C, 49.40; H, 4.70; N, 26.0

Diazotization of 3-amino5,7-dimethylpyrazolo(3,4-d)-pyrimidine-4,6(5H,7H)-dione 1 and coupling with active methylene compounds to give compounds 9, 10 and 11:

General Procedure:

A stirred solution of (0.01 mol) of **1** in 70% nitric acid (d. 1.42; 10 ml) was diazotized at 0-5°C by adding 30% aqueous sodium nitrite solution (20 ml) over 20 minutes. The reaction mixture was stirred for 1 h. To the previous diazotized solution, (0.01 mol) of the appropriate active methylene compounds was added at 0-5°C and the reaction mixture was stirred at room temperature for 2 h. The formed products were filtered off, washed with sodium bicarbonate solution followed by water and recrystallized from the proper solvent as shown in (Table 1).

Table 1:

Cpd. No.	M.P. °C	Yield %	Solvent of Crystallization	Mol. Formula (M.Wt.)	Analysis Calc./(Found) %		
					C	H	N
9	>300	61.1	$CHCl_3$ + EtOH	$C_{13}H_{14}N_8O_5$	43.09	3.8	30.9
				(362)	(43.40)	(3.6)	(30.2)
10	>300	50.5	DMF	$C_{11}H_{10}N_8O_4S$	37.7	2.8	32.0
				(350)	(37.7)	(3.1)	(31.6)
11	>300	50.8	DMF	$C_{17}H_{16}N_8O_3$	53.68	4.2	29.4
				(380)	(53.70)	(3.8)	(29.1)

Synthesis of 7-amino-1,3,5-trimethylpyrazolo-[3,4-d]-pyrimidine-4,6-(3H, 5H)-dione 12a:

A mixture of 0.332 g (1.7 m mol) of **3** and 0.088 ml (1.7 m mol) of methyl hydrazine in absolute ethanol was refluxed for 6 h. The formed product was collected by filtration and recrystallized from methanol to give 0.19 g (54.7%) m.p. 235-238°C.

Analysis:	$C_8H_{11}N_5O_2$ (209)	Required:	C, 45.93; H, 5.26; N, 33.49
		Found:	C, 45.84; H, 5.2; N, 33.2

Synthesis of 7-amino-3,5-dimethyl-1-phenyl pyrazolo-[3,4-d]-pyrimidine-4,6-(3H, 5H)-dione 12b:

A mixture of 0.665 g (3.3 m mol) of **3** and 0.327 ml (3.3 m mol) of phenyl hydrazine in absolute ethanol was refluxed for 1 h. The formed product was collected by filtration and recrystallized from methanol to give 0.54 g (59.8%) m.p. 235-238°C.

Analysis:	$C_{13}H_{13}N_5O_2$ (271)	Required:	C, 57.56; H, 4.79; N, 25.83
		Found:	C, 57.3; H, 4.50; N, 26.0

Synthesis of 7-amino-1-amide-3,5-dimethylpyrazolo-[3,4-d]-pyrimidine-4,6-(3H, 5H)-dione 13:

To a solution of 0.665 g (3.3 m mol) of **3** and 0.446 g (4 m mol) of semicarbazide hydrochloride in methanol, was added dropwise a solution of 0.224 g (4 m mol) of potassium hydroxide in 5 ml water, while the reaction mixture was maintained below 10°C, the mixture was stirred at room temperature for 4 h. The formed product was collected

by filtration and recrystallized from acetone/water to give 0.42 g (53.16%) m.p. 285-287°C.

Analysis:	$C_8H_{10}N_6O_3$ (238)	Required:	C, 40.33; H, 4.20; N, 35.29
		Found:	C, 40.60; H, 4.50; N, 34.9

Synthesis of 7-amino-3,5-dimethyl-1-thioamide-pyrazolo-[3,4-d]-pyrimidine-4,6-(3H, 5H)-dione 14:

To a solution of 0.665 g (3.3 m mol) of **3** and 0.364 g (4 m mol) of thiosemicarbazide in methanol, was added dropwise a solution of 0.224 g (4 m mol) of potassium hydroxide in 5 ml water, while the reaction mixture was maintained below 10°C, the mixture was stirred at room temperature for 6 h. The formed product was collected by filtration and recrystallized from ethanol to give 0.66 g (78.5%) m.p. 230-232°C.

Analysis:	$C_8H_{10}N_6O_2S$ (254)	Required:	C, 37.70; H, 3.90; N, 33.07
		Found:	C, 38.8; H, 3.68; N, 33.2

Synthesis of 5-cyano-1,3-dimethylbarbituric acid 15:

To a solution of 0.165 g (1 m mol) of **3** and 0.052 ml (1 m mol) of malononitrile in methanol, was added dropwise a solution of 0.115 g (1 m mol) of potassium carbonate in 3 ml water. The reaction mixture was refluxed for 1h. The formed product was collected by filtration and recrystallized from methanol, which yielded 0.103 g (53.92%) m.p. 300 °C.

Analysis:	$C_7H_6N_3O_3$ (180)	Required:	C, 46.6; H, 3.30; N, 23.30
		Found:	C, 46.2; H, 3.0; N, 23.1

Synthesis of 7-amino-3,5-dimethyl-isoxazolo-[3,4-d]-pyrimidine-4,6-(3H, 5H)-dione 16:

To a solution of 0.332 g (1.7 m mol) of **3** and 0.139 g (2 m mol) of hydroxylamine hydrochloride in methanol, was added dropwise a solution of 0.112 g (2 m mol) of potassium hydroxide in 5 ml water, while the reaction mixture was maintained below 10°C. The mixture was stirred at room temperature for 6 h. The formed product was collected by filtration and recrystallized from ethanol to give 0.124 g (38.7%) m.p. 240-243°C.

Analysis:	$C_7H_8N_4O_3$	Required:	C, 42.85; H, 8.08; N, 28.57
	(196)	Found:	C, 42.52; H, 7.98; N, 28.21

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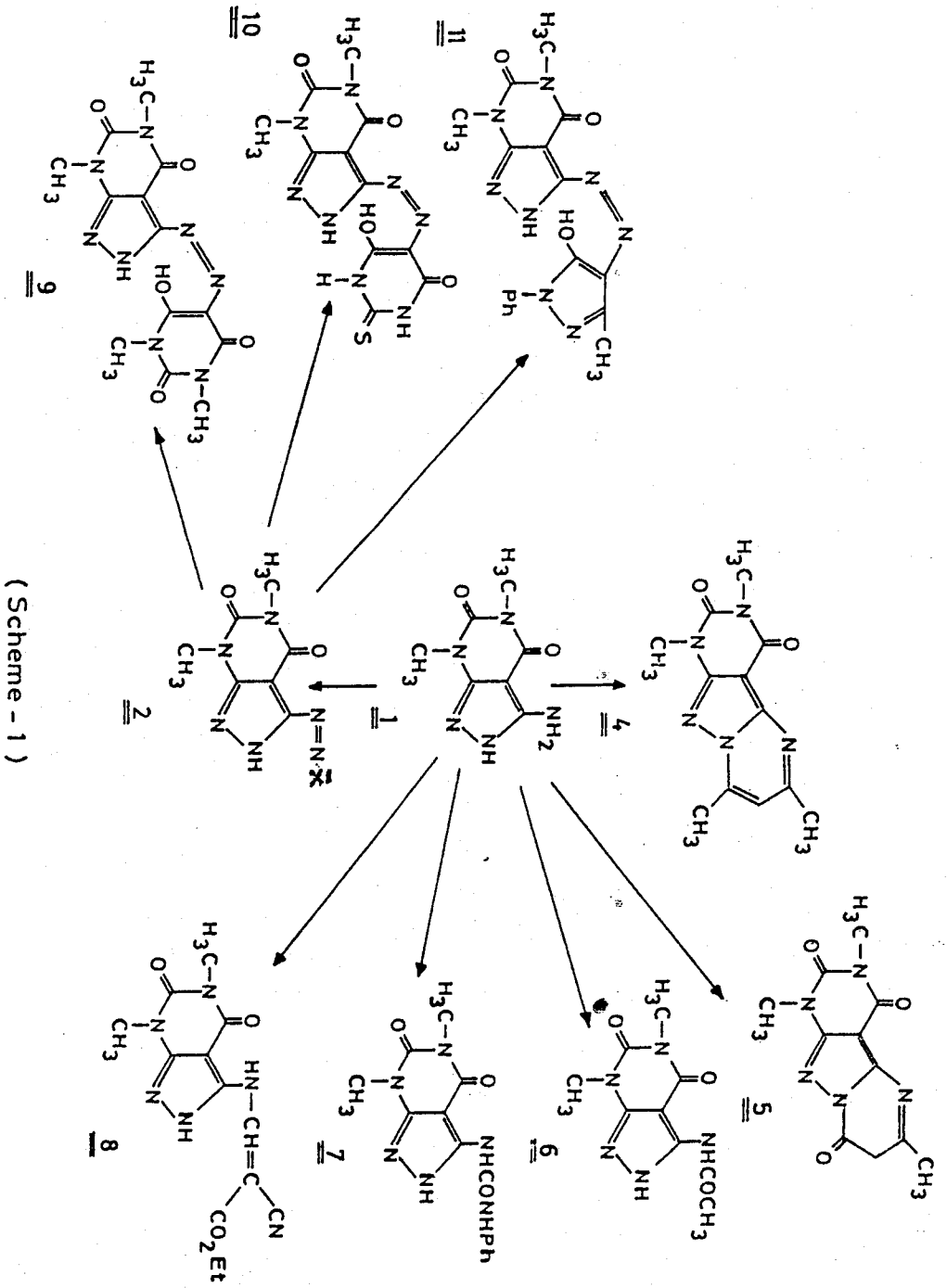
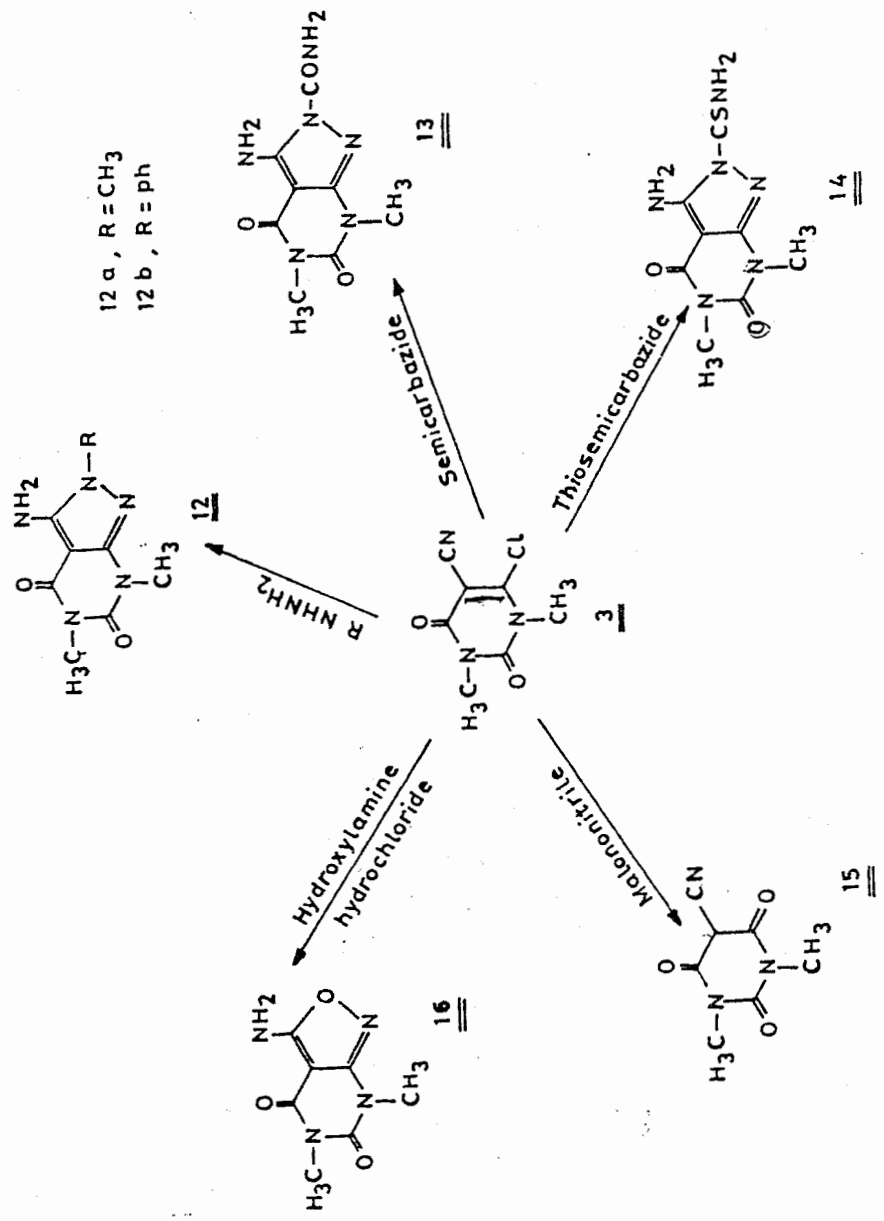


(Scheme - 1)


12 a , R = CH3
12 b , R = ph
12
13
14
15
16
3
Semicarbazide
Thiosemicarbazide
Malononitrile
Hydroxylamine hydrochloride
R NHNH2

(Scheme - 2)

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تخليق بعض مشتقات البريميدين الملتحمة والمتوقع لها نشاط بيولوجى

عبد العليم حسن ، مجدى زهران وحنان معروف

قسم الكيمياء – كلية العلوم – جامعة المنوفية – شبين الكوم – مصر

ملخص البحث :

الهدف من هذا البحث هو تحضير بعض مشتقات البريميدين المختلفة والتى من المتوقع لها تأثير بيولوجى – وكذلك تستخدم أيضا فى عملية الإزدواج مع السكريات المختلفة لتحضير بعض النيوكليوزيدات والنيوكليوتيدات الجديدة . وقد تم ذلك بتفاعل بيرازولو [٣،٤-د] بيريميدين مع أستيل أستون ، إثيل أسيتو أسيتات فى الميثانول ، إثيل أسيتو أسيتات فى أنهيدريد حمض الخليك ، فنيل أيزوسيانات وإيثوكس ميثلين سيانو أسيتات تحت ظروف مختلفة ليعطى مشتقات بيرازولو [٣،٤-د] بيريميدين .
بإجراء تفاعل الدسترة للمركب بيرازولو [٣،٤-د] بيريميدين ليكون ملح الديازنيوم المقابل الذى يتفاعل مع بعض المشتقات التى تحتوى على مجموعة الميثلين النشطة مثل ١،٣-ثنائى ميثيل حمض الباربتيوريك ، حمض الثيوباربتيوريك وأحد مشتقات البيرازولون لتكون مشتقات بيريميدو الهيدرازون المقابلة .
وعند تفاعل ٦-كلورو-٥ سيانو-١،٣-ثنائى مثيل اليوراسيل مع ميثيل هيدرازين ، فنيل هيدرازين ، السيمكربازيد ، الثيوسيمكربازيد ، مالونونتريل والهيدروكسلامين ليعطى مشتقات بيرازولو [٣،٤-د] بيريميدين المختلفة وقد تم إثبات التركيب الكيميائى للمركبات بإستخدام التحليل الدقيق ، الرنين النووى المغناطيسى ، طيف الأشعة تحت الحمراء وطيف الكتلة .