

Synthesis, Radioprotective and Anticancer Activity of Some Novel Pyrazolo[3,4-d]pyrimidines

Thesis Presented by

Sarah Ahmed Mohamed About Magd

B.Sc. in pharmaceutical sciences
Faculty of Pharmacy, Cairo University

Submitted for Partial fulfillment of
Master Degree in Pharmaceutical Sciences
(Pharmaceutical Chemistry)

Under the supervision of

Prof. Dr. Fatma Abd El-Fattah Ragab

Professor of Pharmaceutical Chemistry
Faculty of Pharmacy, Cairo University

Prof. Dr. Mostafa Mohamed Soliman Ghorab

Professor of Applied Organic Chemistry
Drug Radiation Research Department
National Centre for Radiation Research & Technology,
Atomic Energy Authority

Faculty of Pharmacy
Cairo University
(2009)

**تشبيد وتخصير بعض مركبات البيرازولو (٤،٣-د)
بيريميدين الجديدة كمضادات للأورام السرطانية
وخواصها من الإشعاع**

رسالة مقدمة من الصيدلانية

سارة أحمد محمد أبو المجد

بكالوريوس العلوم الصيدلانية
كلية الصيدلة - جامعة القاهرة

للإستيفاء الجزئى

**لدرجة الماجستير فى العلوم الصيدلانية
(كيمياء صيدلانية)**

تحت إشراف

الأستاذ الدكتور / فاطمة محمد الفتاح رجب

أستاذ الكيمياء الصيدلانية
كلية الصيدلة - جامعة القاهرة

الأستاذ الدكتور / مصطفى محمد سليمان خرابج

أستاذ الكيمياء العضوية التطبيقية
قسم البحوث الدوائية الإشعاعية
المركز القومى لبحوث و تكنولوجيا الإشعاع - هيئة الطاقة الذرية

كلية الصيدلة - جامعة القاهرة

٢٠٠٩

Contents

Abstract.....	iv
List of Abbreviations.....	viii
List of Charts.....	x
List of Tables.....	x
I. Introduction.....	1
1 Chemistry of pyrazolo[3,4-d]pyrimidines.....	1
<i>1.1 Synthesis of pyrazolo[3,4-d]pyrimidines.....</i>	<i>1</i>
1.1.1 From non-heterocyclic Compounds.....	1
1.1.2 From pyrazole derivatives.....	2
1.1.3 From pyrimidine derivatives.....	7
<i>1.2 Reactions of pyrazolo[3,4-d]pyrimidines.....</i>	<i>9</i>
2 Biological review	18
2.1 <i>Cancer.....</i>	<i>18</i>
2.1.1 Cancer chemotherapeutic agents	18
2.2 <i>Radiation.....</i>	<i>24</i>
2.2.1 Mechanisms of radioprotection	25
2.2.2 Parameters for evaluation of radioprotective activity	26
2.2.3 Radioprotective compounds	28
2.2.4 Use of Radioprotective Agents in Radiotherapy and Chemotherapy of Cancer.....	31
2.3 <i>Biological significance of pyrazolo[3,4-d]pyrimidines.....</i>	<i>32</i>
2.3.1 Anticancer activity of pyrazolo[3,4-d]pyrimidines.....	32
2.3.2 Other biological activities of pyrazolo[3,4-d]pyrimidines.....	36
II.Aim of the investigation.....	37

III. Discussion 42

Ethyl-5-amino-1-phenyl-1H-pyrazol-4-carboxylate (3).....	47
4-Chloro-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (5).....	47
1,5-Diphenyl-1H-pyrazolo[3,4-d]pyrimidin-4,6(5H,7H)-dione (7)	48
6-Chloro-1,5-diphenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (8).....	48
5-Amino-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (10).....	48
5-Amino-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4,6(5H,7H)-dione (12).....	49
(1-Phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-ylamino)-carboxylic acid derivatives (13a-g) and pyrrolidine-2-carboxylic acid derivative (13h).....	49
7-Phenyl-2,7-dihydro-3H-imidazo[1,2-c]pyrazolo[4,3-e]pyrimidin-3-one derivatives (14a-c)	53
(4-Oxo-1,5-diphenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-carboxylic acid derivatives (15a-c).....	55
1,5 - Diphenyl -5,7-dihydro-1H-imidazo[1,2-a] pyrazolo[4,3-e] pyrimidine -4,8 -dione (16).....	58
Ethyl [(4-oxo-1,5-diphenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-yl)oxy]acetate (17).....	58
6-Hydrazino-1,5-diphenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (18)	59
3,10-Diphenylpyrazolo[3',4': 4,5]pyrimido[1,6-b]pyrazolo[3''',4''': 4'',5''']pyrimido[1'',6''- e][1,2,4,5]tetrazine (20).....	60
5-Amino-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-thione (21).....	62
5-(4- Benzylidene-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl)-1-phenyl-1,5-dihydro-4H- pyrazolo[3,4-d]pyrimidin-4-one (23).....	64
7-Phenyl-3H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-2(7H)-thione (24).....	66
7-({4-[2,2-Bis(methylthio)-1-azavinylsulphonyl]phenyl} amino)-1-phenyl[1,3,4] oxadiazolo [3,2- a]pyrazolo[3,4-d]pyrimidin-4(1H)-one (26).....	66
1-Phenyl[1,3,4]oxadiazolo[3,2-a]pyrazolo[3,4-d]pyrimidin-4(1H)-one (27).....	67
5-Benzyl-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (28).....	68
Ethyl-5-isothiocyanato-1-phenyl-1H-pyrazole-4-carboxylate (29)	69
1-Phenyl-1,10-dihydropyrazolo[3',4':4,5]pyrimido[1,2-a]benzimidazol-4-one (30).....	70
8-Phenyl-5H-1H-pyrazolo[3',4':4,5]pyrimido[1,2-a][3,1]benzoxazin-5,11-dione (31).....	71
1-Phenyl-7-thioxo-1,6,7,8-tetrahydro-pyrazolo[3,4-d][1,2,4] triazolo [1,5-a] pyrimidin-4-one (32) 71	

IV. Experimental..... 73

V. Biological Activity 97

1 In-vitro anticancer activity..... 98

1.1 Methods.....	98
1.2 Results.....	99
1.3 Structure activity relationship.....	101

2	In-vivo radioprotective activity	102
2.1	<i>Experimental design.....</i>	103
2.2	<i>Administration of tested compounds</i>	104
2.3	<i>Sample collection</i>	104
2.4	<i>Analytical procedures</i>	104
2.5	<i>Statistical analysis.....</i>	104
2.6	<i>Effect of the tested compounds and/ or γ-irradiation on lipid peroxidation and antioxidant status.....</i>	106
2.6.1	Glutathione level in blood (GSH).....	106
2.6.2	Superoxide dismutase (SOD) activity in blood.....	106
2.6.3	Lipid peroxidation content (MDA) in plasma	106
VI.	References	108

Abstract

A series of new pyrazolo[3,4-d]pyrimidine derivatives was synthesized. Most of the newly synthesized target compounds were subjected to in-vitro anticancer screening against Ehrlich Ascites Carcinoma cells. Also, some of these new compounds were evaluated for their radioprotective activity.

The thesis includes the following parts:

Introduction:

This part includes a brief literature review on different classes of anticancer and radioprotective agents, and anticancer activity of pyrazolo[3,4-d]pyrimidine regarding their mechanisms of action. In addition, the different methods for the synthesis of pyrazolo[3,4-d]pyrimidine derivatives are discussed.

Aim of the present investigation:

This part includes the biological bases on which the synthesized compounds were designed.

Discussion:

This part deals with the discussion of the experimental methods adopted for the synthesis of the designed compounds, as well as different analytical methods used for identification and verification of the synthesized compounds.

Experimental:

This part describes the practical procedures used for the synthesis of twenty seven new final compounds, their elemental analysis and spectral data (IR, ¹H-NMR and mass spectra).

The thesis comprises the synthesis of the following *reported* and *new* compounds:

a. Known intermediates:

- Ethyl-2-cyano-3-ethoxyacrylate (2)
- Ethyl-5-amino-1-phenyl-1H-pyrazol-4-carboxylate (3)
- 1-Phenyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4)
- 4-Chloro-1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidine (5)
- Ethyl 5-[(anilincarbonyl)amino]-1-phenyl-1H-pyrazole-4-carboxylate (6)
- 1,5-Diphenyl-1H-pyrazolo[3,4-*d*]pyrimidin-4,6(5*H*,7*H*)-dione (7)
- 6-Chloro-1,5-diphenyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (8)
- 5-[(Ethoxymethylene)amino]-1-phenyl-1H-pyrazol-4-carboxylate (9)
- 5-Amino-1-phenyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (10)
- 5-Amino-1-phenyl-1*H*-pyrazol-4-carbohydrazide (11)
- 5-Amino-1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-4,6(5*H*,7*H*)-dione (12)

b. New intermediate:

- Ethyl-5-isothiocyanato-1-phenyl-1H-pyrazol-4-carboxylate (29)

c. New final compounds:

- (1-Phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)-acetic acid (13a)
- 2-(1-Phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)-propanoic acid (13b)
- 3-Methyl-2-(1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)-butanoic acid (13c)
- 4-Methyl-2-(1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)-pentanoic acid (13d)
- 4-Methylthio-2-(1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)-butanoic acid (13e)

- 3-Phenyl-2-(1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-ylamino)-propanoic acid (13f)
- 3-(4-Hydroxyphenyl)-2-(1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-ylamino) propanoic acid (13g)
- 1-(1-Phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)-pyrrolidin-2-carboxylic acid (13h)
- 2-Isobutyl-7-phenyl-2,7-dihydro-3H-imidazo[1,2-c]pyrazolo[4,3-e]pyrimidin-3-one (14a)
- 2-[2-(Methylthio)ethyl]-7-phenyl-2,7-dihydro-3H-imidazo[1,2-c]pyrazolo[4,3-e]pyrimidin-3-one (14b)
- 2-Benzyl-7-phenyl-2,7-dihydro-3H-imidazo[1,2-c]pyrazolo[4,3-e]pyrimidin-3-one (14c)
- (4-Oxo-1,5-diphenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-acetic acid (15a)
- 2-(4-Oxo-1,5-diphenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-propanoic acid (15b)
- 3-Methyl-2-(4-oxo-1,5-diphenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-butyric acid (15c)
- 1,5-Diphenyl-5,7-dihydro-1H-imidazo[1,2-a]pyrazolo[4,3-e]pyrimidin-4,8-dione (16)
- Ethyl [(4-oxo-1,5-diphenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-6-yl)oxy]acetate (17)
- 6-Hydrazino-1,5-diphenyl-1,5-dihydro-pyrazolo[3,4-d]pyrimidin-4-one (18)
- 3,10-Diphenylpyrazolo[3',4': 4,5]pyrimido[1,6-b]pyrazolo[3'',4''': 4'',5''']pyrimido[1'',6''-e][1,2,4,5]tetrazine (20)
- 5-Amino-1-phenyl-1,5-dihydro-pyrazolo[3,4-d]pyrimidin-4-thione (21)
- 5-(4-Benzylidene-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl)-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (23)

- 7-Phenyl-3H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-2(7H)-thione (24)
- 7-(4-[2,2-Bis(methylthio)-1-azavinylsulphonyl]phenyl)amino)-1-phenyl[1,3,4]oxadiazolo[3,2-a]pyrazolo[3,4-d]pyrimidin-4(1H)-one (26)
- 1-Phenyl[1,3,4]oxadiazolo[3,2-a]pyrazolo[3,4-d]pyrimidin-4(1H)-one (27)
- 5-Benzyl-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (28)
- 1-Phenyl-1,10-dihydropyrazolo[3',4':4,5]pyrimido[1,2-a]benzimidazol-4-one (30)
- 8-Phenyl-5H-11H-pyrazolo[3',4':4,5]pyrimido[1,2-a][3,1]benzoxazin-5,11-dione (31)
- 1-Phenyl-7-thioxo-1,6,7,8-tetrahydro-pyrazolo[3,4-d][1,2,4]triazolo[1,5-a]pyrimidin-4-one (32)

Biological activity:

Twenty four new compounds were evaluated for their in-vitro anticancer activity and three compounds were evaluated for their in-vivo radioprotective activity. The results are presented and discussed.

References:

This part includes 123 references.

Arabic summary

List of abbreviations

ADA: Adenosine deaminase
CMC: Carboxy methyl cellulose
COX-2: Cyclooxygenase 2
CDKs: Cyclin Dependent Kinases
dATP: Deoxyadenosine triphosphate
dCTP: Deoxycytidine triphosphate
dGTP: Deoxyguanosine triphosphate
dTTP: Deoxythymidine triphosphate
DEAD: Diethyl azodicarboxylate
DHFR: Dihydrofolate reductase
DMF: Dimethylformamide
DMSO: Dimethyl sulfoxide
DNA deoxyribonucleic acid
dTMP: Deoxythymidine monophosphate
dUMP: Deoxyuridylic acid monophosphate
EAC: Ehrlich Ascites Carcinoma
EGF-R: Epidermal growth factor receptor
FTase: Farnesyl transferase
GDP: Guanosine diphosphate
GSH: Glutathione
GTP: Guanosine triphosphate
Gy: Gray
HBTU: Benzotriazole tetramethyl uronium hexafluorophosphate
¹H-NMR: Proton nuclear magnetic resonance
i.p.: Intraperitoneal
IC₅₀: Inhibitory concentration causing 50% mortality in net cells.
LPx: Lipid peroxide
m-CPBA: *meta*-chloro perbenzoic acid

MDA: malondialdehyde
MEA: 2-Mercaptoethylamine
mRNA: Messenger ribonucleic acid
NCRRT: National Centre for Radiation Research and Technology
PKB: Protein kinase B (AKT kinase)
PTKs: Protein tyrosine kinases
ROS: Reactive oxygen species (ROS)
SOD: Superoxide dismutase
SPS: Solid phase synthesis
SRC: A family of proto-oncogenic tyrosine kinases
TEA: Triethylamine
TEOF: Triethyl orthoformate
THF: Tetrahydrofuran
TFA: Trifluoroacetic acid
WHO: World Health Organization

List of charts

Chart 1: Mass fragmentation pattern of compound 13d.....	51
Chart 2: Mass fragmentation pattern of compound 13h.....	52
Chart 3: Mass fragmentation pattern of compound 14b.....	54
Chart 4: Mass fragmentation pattern of compound 15a.....	56
Chart 5: Mass fragmentation pattern of compound 15b.....	57
Chart 6: Mass fragmentation pattern of compound 20.....	61
Chart 7: Mass fragmentation pattern of compound 21.....	63
Chart 8: Mass fragmentation pattern of compound 23.....	65
Chart 9: Mass fragmentation pattern of compound 28.....	69
Chart 10: Mass fragmentation pattern of compound 32.....	72

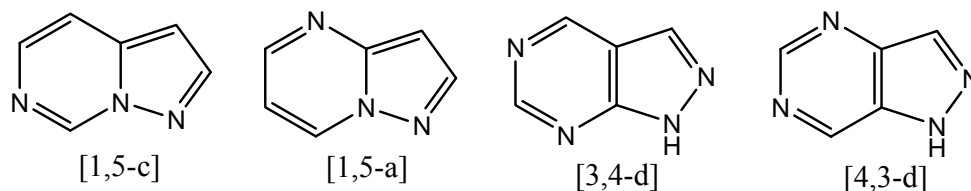
List of tables

Table 1: Physico-chemical data of compounds (13a-h).....	75
Table 2: Physico-chemical data of compounds (14a-c).....	79
Table 3: Physico-chemical data of compounds (15a-c).....	81
Table 4: In-vitro cytotoxic activity for some of the new compounds.....	99
Table 5: Effect of compounds 13e , 13h and 21 administration on blood glutathione (GSH) content, activity levels of superoxide dismutase (SOD) and plasma lipid peroxide concentrations (LPx) of normal and irradiated mice.	105

I. Introduction

1 Chemistry of pyrazolo[3,4-d]pyrimidines

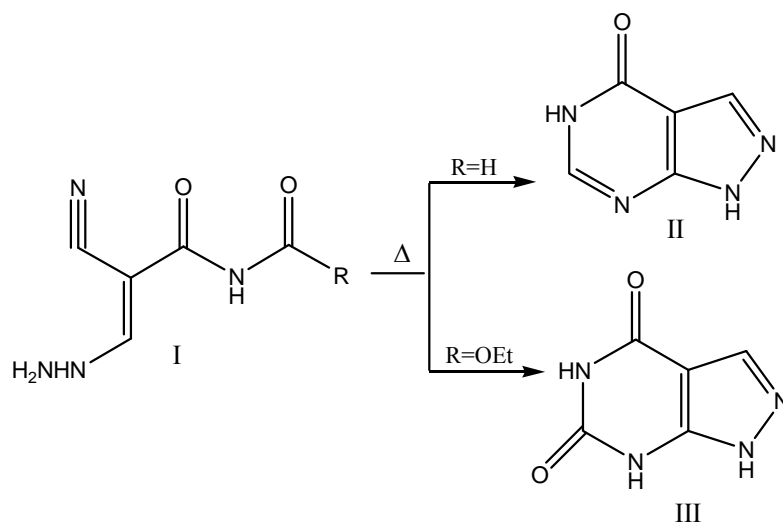
Fusion of a pyrazole ring to a pyrimidine nucleus gives rise to four positional isomers of pyrazolopyrimidines which are: pyrazolo[1,5-c]pyrimidine, pyrazolo[1,5-a]pyrimidine, pyrazolo[3,4-d]pyrimidine and pyrazolo[4,3-d]pyrimidine. This study focuses mainly on the synthesis of some new pyrazolo[3,4-d]pyrimidine derivatives.



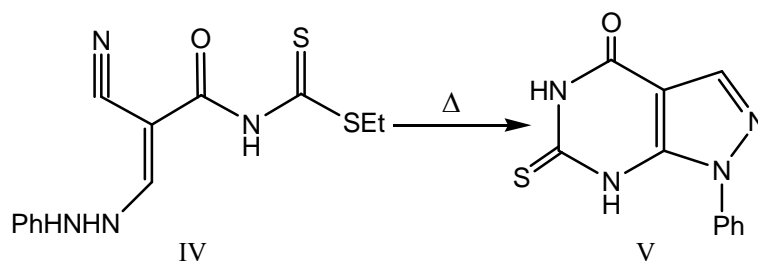
1.1 Synthesis of pyrazolo[3,4-d]pyrimidines

1.1.1 From non-heterocyclic Compounds

Heating the hydrazine derivative **I** at 150 °C gave directly the pyrazolo[3,4-d]pyrimidine in an example of an important synthesis of allopurinol **II** and oxyallopurinol **III**¹.



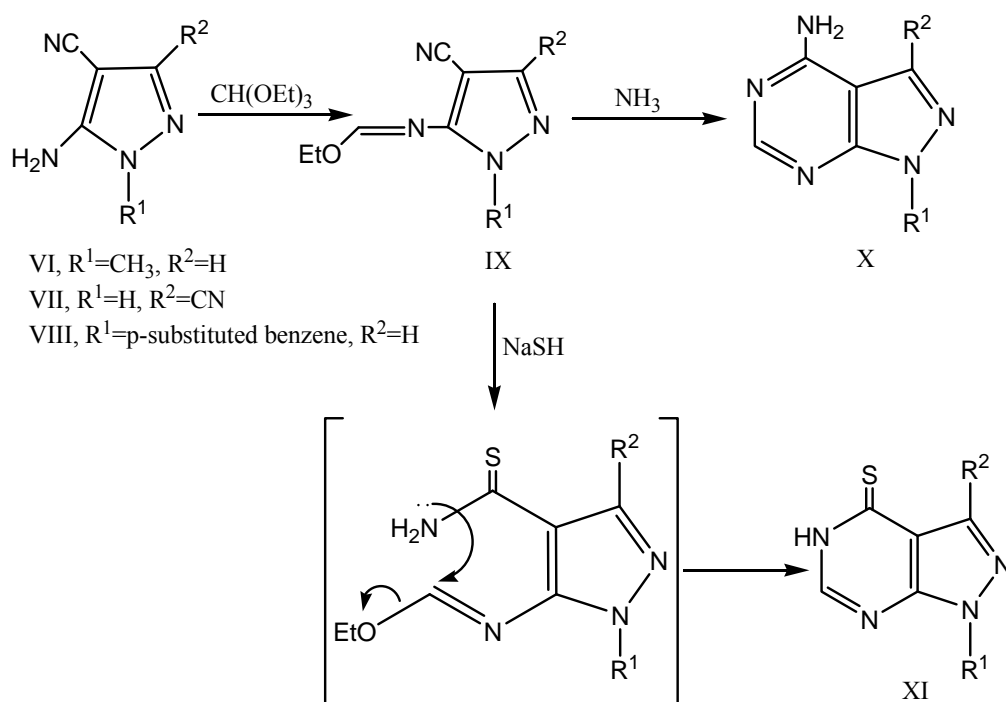
The dithiocarbamate derivative **IV** could also be converted directly into the pyrazolopyrimidine **V** by heating¹.



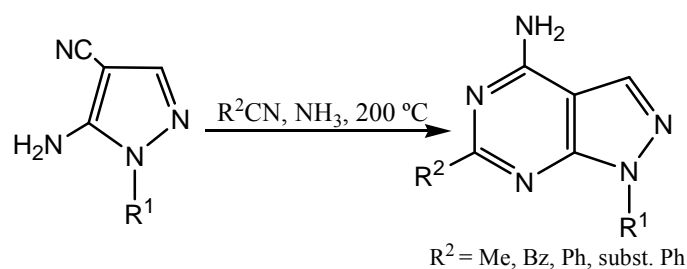
1.1.2 From pyrazole derivatives

1.1.2.1 From o-aminopyrazolo nitriles

Reaction of o-aminopyrazolo nitriles **VI**, **VII**, and **VIII** with triethyl orthoformate in acetic anhydride, gave the imidates **IX** (ethoxymethylene amino derivatives) which were cyclised with ammonia, to give the respective pyrazolo[3,4-d]pyrimidines **X**²⁻⁴. On the other hand, treatment of the imidates **IX** with sodium hydrosulfide hydrolyzed the nitrile group to the thioamides which spontaneously underwent ring closure to thiopurinol derivatives **XI**⁵.



Also, o-aminopyrazolo carbonitriles **VI**, **XII**, **XIII** reacted with simple nitriles to give aminopyrazolopyrimidines **XIV**⁶.



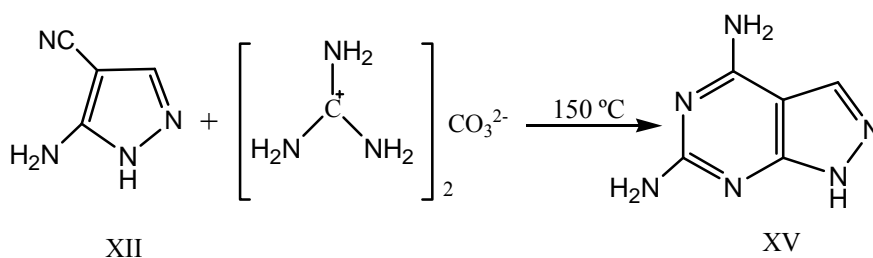
VI, $R^1 = \text{CH}_3$

XII, $R^1 = \text{H}$

XIII, $R^1 = \text{Ph}$

XIV

On the other hand, Reaction of the carbonitrile **XII** with guanidine carbonate, yielded a pyrimidine nucleus with an additional amino group **XV**⁷.



Pyrazolo[3,4-d]pyrimidines **XVI** and **XVII** were obtained by the reaction of o-aminopyrazolo nitrile **XII** with formamide and urea or thiourea respectively⁸. Moreover, this reaction could be applied to o-aminopyrazolo amides and o-aminopyrazolo esters yielding the pyrazolo[3,4-d]pyrimidine-4-one derivatives⁸⁻

11

