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

Thesis Presented by

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٢٠٠٩

Contents

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, ^{1}H-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-[(anilinocarbonyl)amino]-1-phenyl-1H-pyrazole-4-carboxylate (6)
- 1,5-Diphenyl-1H-pyrazolo[3,4-d]pyrimidin-4,6(5H,7H)-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-4H-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(5H,7H)-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-ylmino)-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 hexaflourophosphate

^{1}H-NMR: Proton nuclear magnetic resonance

i.p.: Intraperitoneal

IC_{50}: 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: Triflouroacetic acid

WHO: World Health Organization

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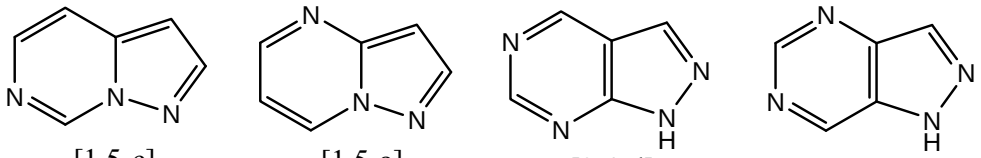
I. Introduction

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

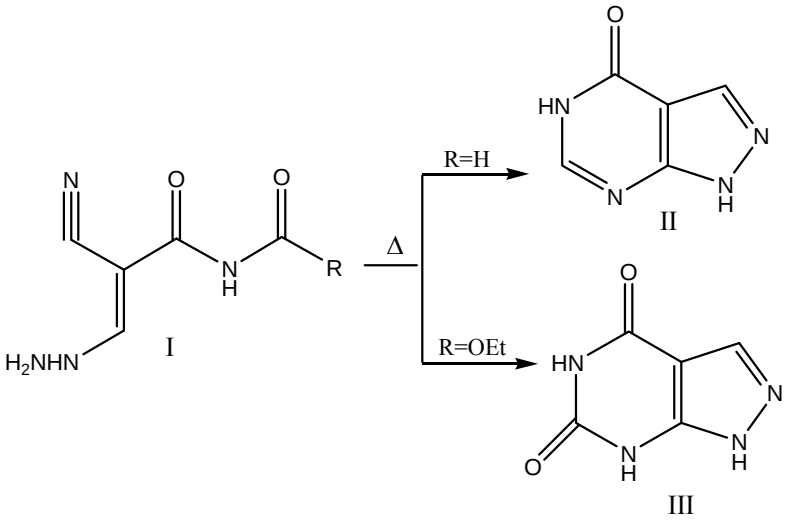
Fusion of a pyrazole ring to a pyrimidine nucleus gives rise to four positional isomers of pyrazolopyrimidines which are: pyrazolo[1,5-c]pyrimidine, pyrazolo[1,5-a]pyrimidine, pyrazolo[3,4-d]pyrimidine and pyrazolo[4,3-d]pyrimidine. This study focuses mainly on the synthesis of some new pyrazolo[3,4-d]pyrimidine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



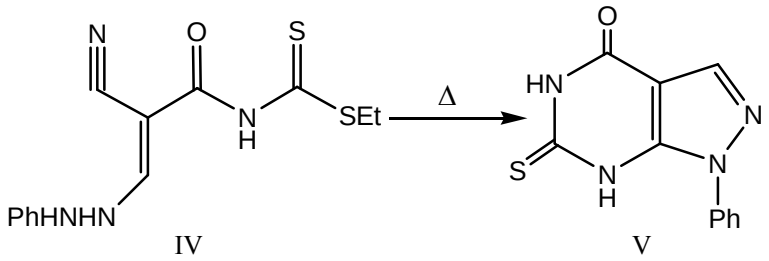
1.1 Synthesis of pyrazolo[3,4-d]pyrimidines

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 heating[1].

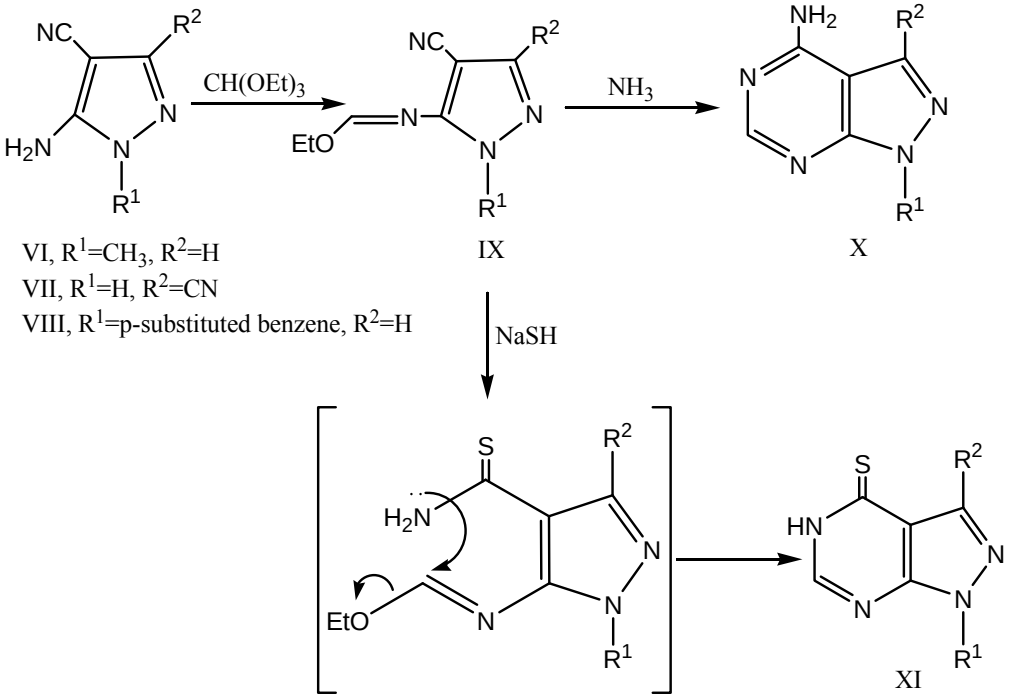


IV V

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].

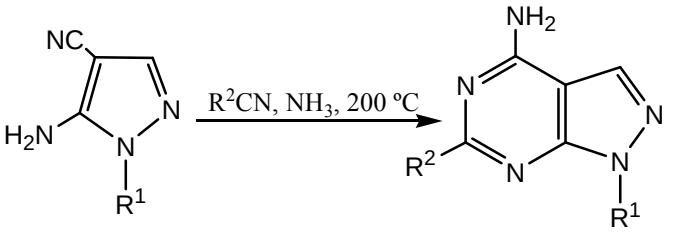


VI, R^1=CH_3, R^2=H
VII, R^1=H, R^2=CN
VIII, R^1=p-substituted benzene, R^2=H

IX X

XI

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

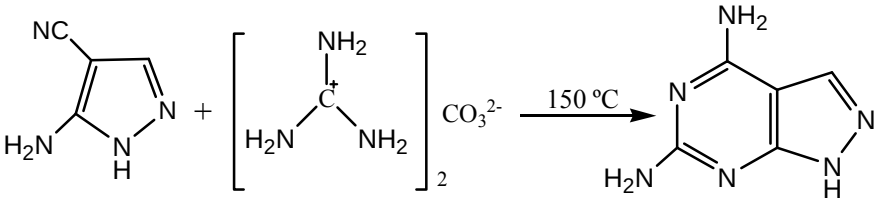


R^2 = Me, Bz, Ph, subst. Ph

VI, R^1 =CH_3
XII, R^1 =H
XIII, R^1 =Ph

XIV

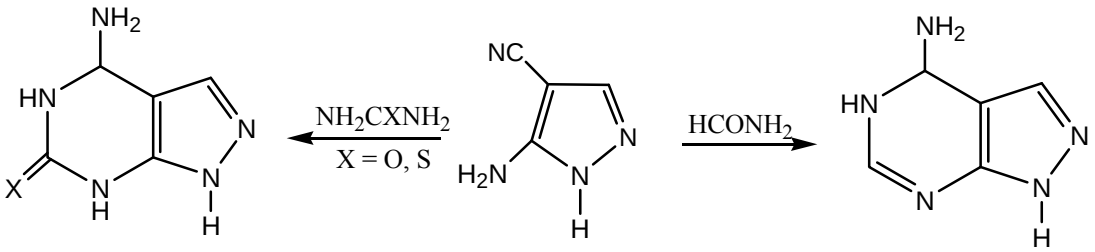
On the other hand, Reaction of the carbonitrile **XII** with guanidine carbonate, yielded a pyrimidine nucleus with an additional amino group **XV**[7].



XII

XV

Pyrazolo[3,4-d]pyrimidines **XVI** and **XVII** were obtained by the reaction of o-aminopyrazolo nitrile **XII** with formamide and urea or thiourea respectively[8]. Moreover, this reaction could be applied to o-aminopyrazolo amides and o-aminopyrazolo esters yielding the pyrazolo[3,4-d]pyrimidine-4-one derivatives[8-11].



XVII

XII

XVI