SYNTHESIS OF NEW 5-*N*-PYRAZOLYL AMINO ACIDS, PYRAZOLOPYRIMIDINES AND PYRAZOLOPYRIDINES DERIVATIVES

A. M. Shalaby, O. A. Fathalla, E. M. M. Kassem, and M. E. A. Zaki*

National Research Centre, Dokki, Cairo, Egypt
e-mail : mzaki@nrc.sci.eg

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Abstract

Reaction of ketene derivatives (**1a,b**) on treatment with 4-amino-1-phenyl-2,3-dimethyl pyrazolin-5-one gives compounds (**2a,b**) which react with hydrazine to afford 1-phenyl-2,3 dimethyl-4-[3-(5-amino-4-substituted)-1*H*-pyrazolylamino]pyrazolin-5-one (3a,b).

Compounds (**3a,b**) on treatment with Boc. amino acids derivatives, acetic anhydride, ethyl acetoacetate and aldehydes afforded (**4a-h**), (**5a-h**), (**6-10**), (**11a,b**), respectively. Interaction of (**9**) or (**10**) with acetohydrazide, hydrazines, and then with p-bromobenzaldehyde afforded compounds (**13**), (**14a,b**) and (**15**), respectively. Interaction of (**3a**) with thioglycollic acid afforded pyrazolopyridines derivatives (**16**).

Introduction

Amino acids and peptides play an outstanding role as growth factors, hormones, ionophores, antibiotics, immune peptides and toxins[1,2]. Substantial literature evidence has been accumulated that amino acids and peptides, being natural and multifunctional, their conjugates with biologically active agents which are generally synthetic organics are supposed to be more potent and particularly less toxic than their parent compounds[3,4].

Pyrazolo[3,4-*d*]pyrimidines are of considerable chemical and pharmacological importance as purine analogues[5,6], and have antitumor and antileukemic activities. Pyrazolo[1,5-*a*]pyrimidines are also purine analogues and as such have useful properties as antimetabolites in purine biochemical reaction [7-9]. Moreover, biological activity[10-12] of pyrazole and its derivatives is well known. antischistosomal activity has been observed for derivatives of this ring system[13] making these fused azoles excellent candidates as potential drugs for Schistosomiasis.

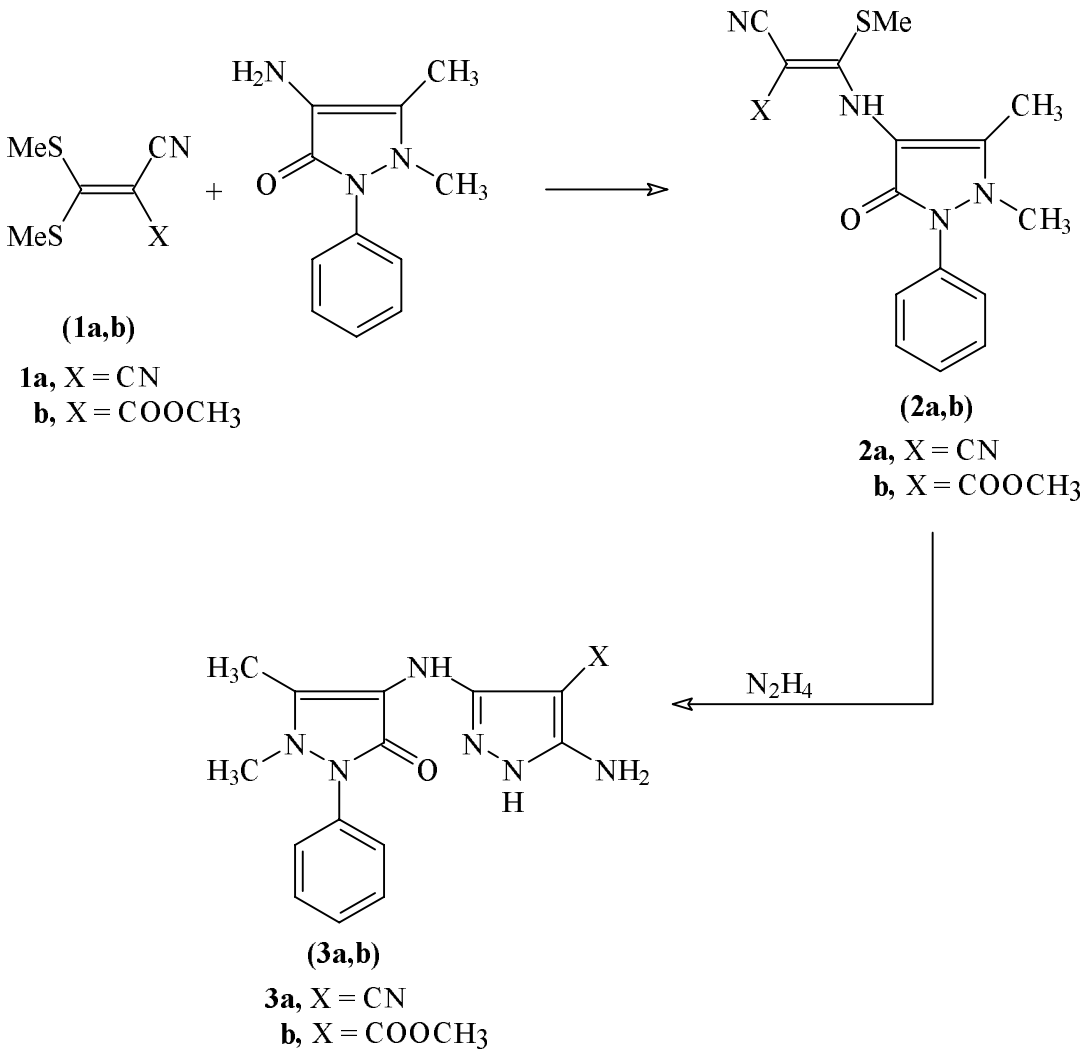
Results and Discussion

In the present work, 5-*N*-pyrazolyl amino acids were conjugated with pyrazolopyrimidines and pyrazolopyridines with the intention to obtain new compounds with biological activities.

Five protected proteinogenic amino acids were selected as different classes of amino acids, namely: Boc-L-valine, Z-L-leucine, Boc-L-threonine (OBzL), Boc-L-phenyl alanine, and Boc-L-histidine. All these amino acids have pharmacological activities in many aspects and in nutrition[14-16]. *tert*-Butyloxycarbonyl (Boc) and carbobenzoxy (Z) are protecting groups used as N-terminal substituents of amino acids.

In the present work, 5-amino-3-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)amino]-1*H*-pyrazole-4-carbonitrile (**3a**)[17] and methyl-5-amino-3-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)amino]-1*H*-pyrazol-4-carboxylate (**3b**) were synthesized by the reaction of hydrazine hydrate with 2-[[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)amino](methyl-sulfanyl)methylene]malononitrile (**2a**)[17] and methyl(2-*E*)-2-cyano-3-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)amino]-3-(methylsulfanyl)-2-propenoate (**2b**) obtained by the reaction of methyl-2-cyano-3,3-bis (methyl-sulfanyl) acrylate (**1b**) with 4-amino-1-phenyl-2,3,dimethyl pyrazolin-5-one, respectively (Scheme 1).

1-Phenyl-2,3-dimethyl-4- [3-(5-substituted amino-4-cyano)-1*H*-pyrazolyl-amino]pyrazolin-5-one derivatives, also 1-phenyl-2,3-dimethyl-4-[-3-(5-substituted amino-4-carbomethoxy)-1*H*-pyrazolylamino]pyrazolin-5-one, (**4a-h**), (**5a-h**), (**6**), respectively, were prepared by direct coupling of compound (**3a,b**) with *N*-terminal protected amino acids namely L-valine, L-threonine, L-phenylalanine and L-histidine using t-butyloxycarbonyl (Boc) and carbobenzoxy (Z) as protecting groups. Coupling took place using (DCC)[19,20] and *N*-hydroxybenzotriazole (HOBT)[21] as a catalyst to afford compounds (**4a-h**). Deprotection of compounds (**4a-h**) took place to furnish compounds (**5a-h**). 1-Phenyl-2,3-dimethyl-4-[3-(5-substituted amino-4-cyano)-1*H*-pyrazolylamino]pyrazolin-5-one dipeptide derivative (**6**) was prepared by coupling of compound (**5c**) with carbobenzoxy L-leucine via the active ester method using *N*-hydroxysuccinimide[22] (HoSu) and dicyclohexylcarbodiimide (DCC) as coupling reagent (Scheme 2).

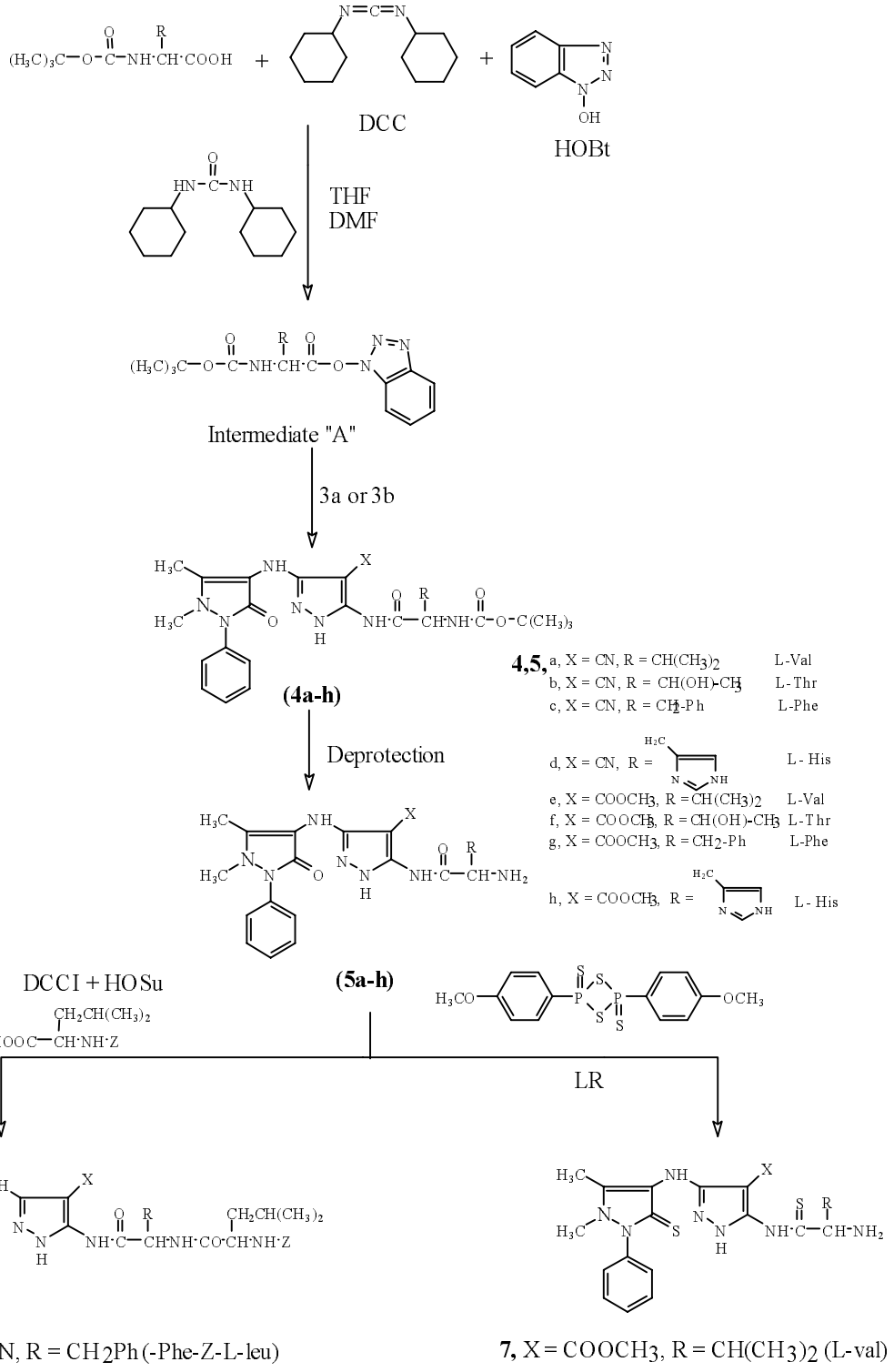


Scheme 1

Thionation of (**5e**) by using Lawesson's reagent (L.R.) afforded thio-analogs (**7**), which are difficult to prepare from their precursor according to thermal oxidation of sulfur. 1-phenyl-2,3-dimethyl-4-[3-(5-thioamido-L-valine-4-carbomethoxy)-1*H*-pyrazolylamino]pyrazolin-5-thione (**7**) was accompanied by a general deshielding effect on the amino acid residues, particularly the α-CH, and thioamide NH protons were shifted downfield by about 0.5-1.0 ppm relative to their amide analogs (Scheme 2).

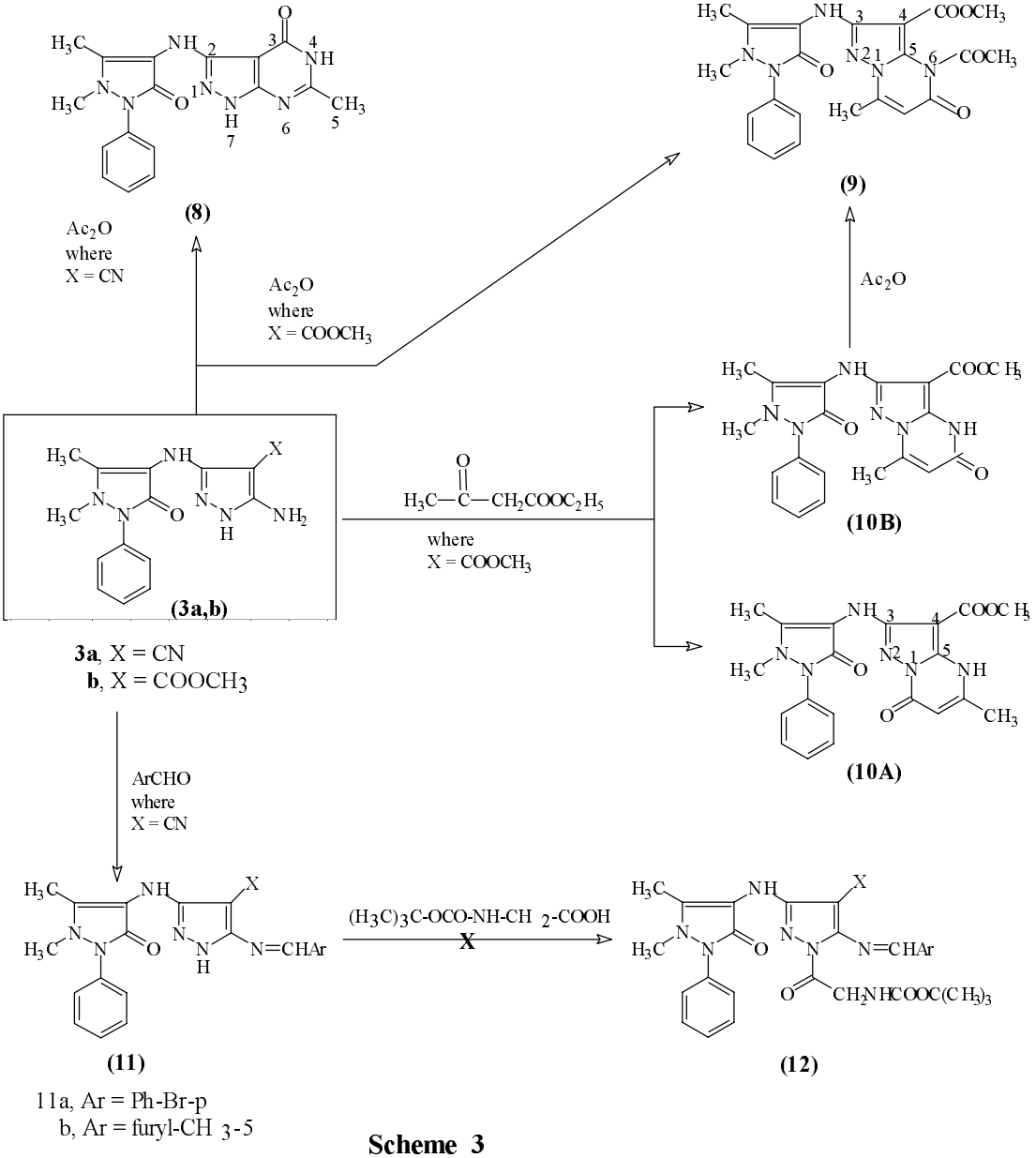
Reaction of compound **3a** with acetic anhydride afforded 1-phenyl-2,3-dimethyl -4-[3-(methylpyrazolo[3,4-*d*])pyrimidine-3-one)-1*H*-pyrazolylamino]pyrazolin-5-one, **8** in good yield, while the interaction of compound **3b** with acetic anhydride yielded a theoretically possible isomeric structure **9** by condensation. Structure of **9** was proven by ^{1}H NMR

spectrum, which revealed signals for pyrimidine CH and CH_3 groups at 7.1 and 2.4 ppm as singlets. Moreover, structure **9** was further proven by the presence of the amide and CO absorptions at 1615 and 1680 cm^{-1}, respectively.



Scheme 2

The basic strategy to synthesize pyrazolo[1,5-*a*]pyrimidines is based on the cyclocondensation of aminopyrazoles with β-ketoesters, β-diketones and β-ketoaldehydes but often these reactions result in regiomeric mixture due to competitive reactivity of 1,3-nucleophilic centers. The regioselectivity in these reactions is often achieved to greater extent by the use of appropriate solvent. Acetic acid was considered as most suitable solvent for the condensation-cyclization reaction to achieve isomeric



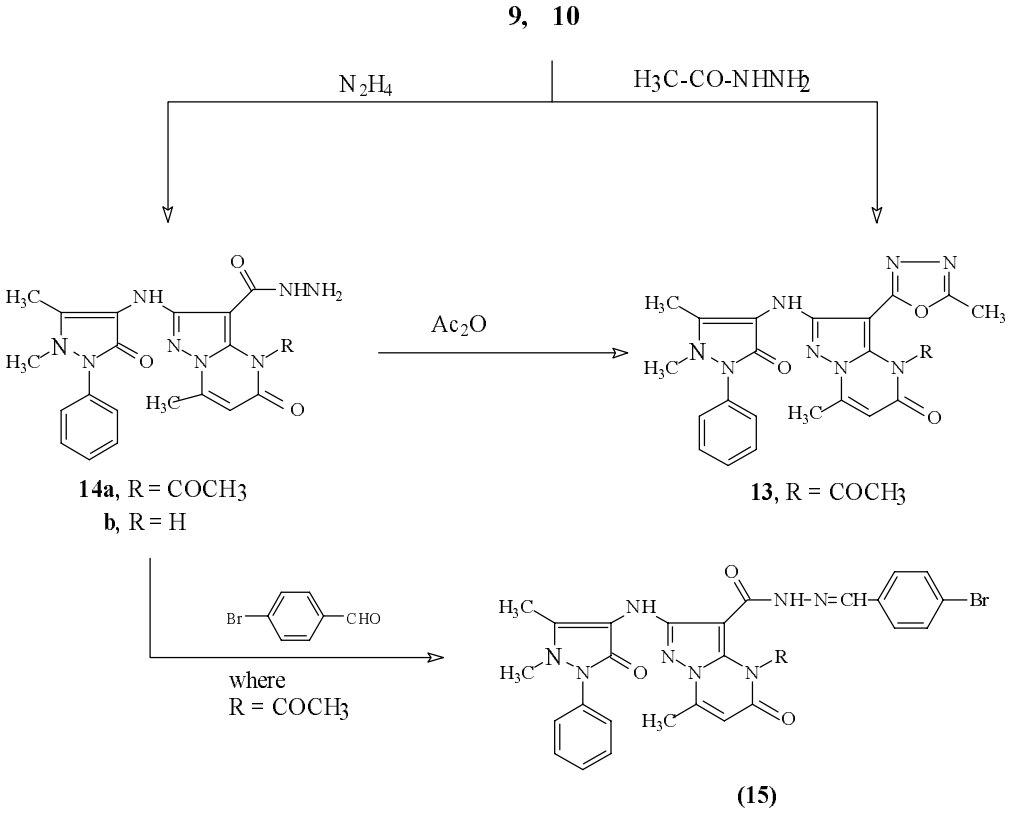
Scheme 3

selectivity. Interaction of **3b** with ethylacetoacetate in acetic acid afforded two theoretically possible isomeric structures **10A, 10B**. Structure **10A** was readily excluded

based on ^{1}H NMR spectrum which revealed signals for pyrimidine CH and CH_3 groups at 2.4 and 7.0 ppm as singlets, respectively. This spectrum corresponds to **10B**, 1-phenyl-2,3,dimethyl-4-[3-(3-carbomethoxy-4*H*-5-methylpyrazolo[1,5-*a*)-1*H*-pyrazolylamino]pyrazolin-5-one. The structure was proven by chemical transformation by acetylation into **9** in moderate yield.

Also, compound (**3a**) reacted with different aldehydes, namely *p*-bromo-benzaldehyde and 5-methylfurfural to give compounds (**11a,b**) respectively (Scheme 3), while coupling of (**11a**) with *N*-terminal protected acid to afford 1-*N*-pyrazolyl protected amino acid (**12**) failed.

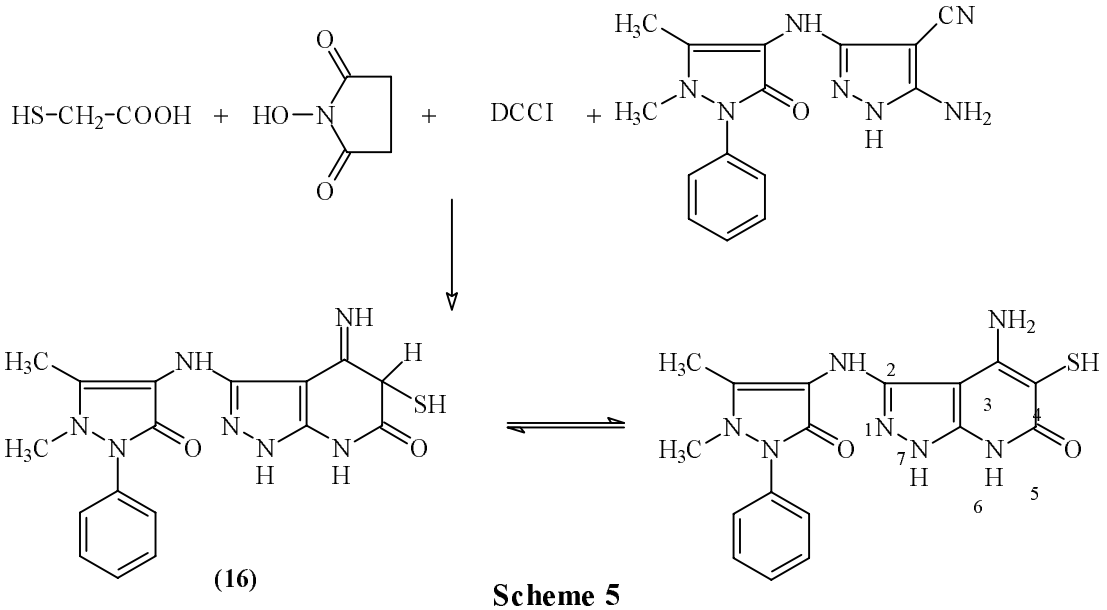
In continuation to our programe[17,18,24-26] for synthesis of new heterocycles based on pyrazolopyrimidines of biological interest, we report herein the interaction of compoud **9** with acetohydrazide to yield compound **13**. The formation of 2-(4-antipyryl)-3-(2-methyl-1,3,4-oxadiazolyl)-7-methylpyrazolo[3,4-*d*]-pyrimidine-5-one (**13**) took place by elimination of methanol and water (Scheme 4). The structure of compound **13** was confirmed chemically by transformations of compounds **9** and/or **10** with hydrazine to afford (**14a,b**) respectively (Scheme 4).



Scheme 4

Compounds **14a,b** were acylated and cyclized by acetic anhydride to afford **13**. Also, compound **14a** reacted with *p*-bromobenzaldehyde to give compound **15** (Scheme 4).

The cyclocondensation of β-keto ester such as ethyl acetoacetate with 1,3-disubstituted-5-aminopyrazole has been previously studied by several investigators to afford pyrazolo[3,4-*d*]pyrimidine[27]. The interaction of 4,5-disubstituted pyrazoles with thioglycolic acid was not reported before. For this, we reported herein the interaction of (**3a**) with thioglycolic acid to afford 3-mercapto-4-amino-5-(4-antipyryl-amino)pyrazolo[3,4-*b*]pyridine-5(1*H*)-one (**16**) by using DCC and HoSu (Scheme 5).



(16)

Scheme 5

Most of the newly synthesized compounds are subjected to pharmacological investigation and will be published elsewhere.

Experimental

Melting points are uncorrected. Microanalyses were carried out in the Microanalytical Centre, Cairo University, Egypt. IR (KBr) spectra were measured on a Karl Zeiss IMP 16 spectrophotometer. ^{1}H-NMR spectra were measured by using Jeol spectrometer EX-270. 1H chemical shifts are reported in δ downfield from internal Me_4Si. Mass spectra were recorded on a MS 30 (AEL) with electron impact ionisation at 70 eV. All thin layer chromatography (TLC) was done on silica gel on aluminum sheets ($60F_{245}$-Merck) using ninhydrine and starch reagents for the detection of amino acid derivatives.

3-(4-Antipyrylamino)-4-cyano-5-aminopyrazole (**3a**) was synthesized according to the procedure described before[17].

1-Phenyl-2,3-dimethyl-4-[5-amino-4-(carbomethoxy)-1*H*-pyrazolylamino]pyrazolin-5-one (3b) :

A mixture of (**2b**)[17] (0.001 mole) and hydrazine hydrate (0.0012 mol) was heated in (50 ml) ethanol under reflux for 3 hr., then poured onto water. The solid precipitate was collected and recrystallized from ethanol to afford (3b), 80%, m.p.195-7 °C, Found: C 56.29, H 5.32, N 24.25, $C_{16}H_{18}N_6O_3$ (342.40), required: C 56.12, H 5.31, N 24.33. ^{1}H-NMR (DMSO-d_6): 2.3 (s, 3H, C-CH_3), 3.2 (s, 3H, N-CH_3), 3.8 (s, 3H, OCH_3), 4.8, 7.8 (s, 2H. 2NH, exchangeable with D_2O), 6.3 (b, 2H, NH_2, exchangeable with D_2O), 7.2-7.5 (m, 5H, Ar-H).

1-Phenyl-2,3-dimethyl-4-[3-(5-substituted amino-4-cyano)-1*H*-pyrazolylamino] pyrazolin-5-one derivatives (4a-d):

General procedure :

A solution of DCC (0.0015 mol) in dimethylformamide was added portionwise to a stirred solution of N-protected amino acid (0.0015 mol) and *N*-hydroxybenzotriazole (0.0015 mol) in 10 ml dimethylformamide at 0-5 °C. A cold solution of (3a) (0.0015 mol) in 10 ml dimethylformamide was added at the same temperature with adjustment of the pH to about 8. The mixture was stirred at at 0 C° for about 3 hr., finally at room temperature for about 48 hrs., Drops of glacial acetic acid were added, the precipitate dicyclohexyl urea was filtered off. The reaction mixture was then poured over ice, stirred well and the precipitate formed was extracted with ethyl acetate several times. The organic layer was separated and washed with sodium hydrogencarbonate (0.5N, 3x10 ml), water (3x10 ml), $KHSO_4$ (0.5N, 3x10 ml) and water (3x10 ml).The solvent was evaporated to dryness. The new synthesized compounds were chromatographically purified to afford (**4a-d**).

4a: 63%, m.p. 121-23 °C, $C_{25}H_{32}N_8O_4$ (508.58), Found: C 59.19, H 6.11, N 22.10, required: C 59.16, H 6.16, N 22.08. IR (γ/cm^{-1}): 3310 (NH), 3290 (NH), 2220 (CN), 1750 (CO, ester), 1630 (CO, amide). ^{1}H-NMR ($CDCl_3$): 0.78-0.81 (d, 3H, γ-CH_3), 0.94-0.97 (d, 3H, γ'-CH_3), 1.43 (s, 9H, 3xCH_3), 2.33 (m, 1H, β-CH), 2.37 (s, 3H, C-CH_3), 3.05 (s, 3H, N-CH_3), 5.12 (m, 1H, α-CH), 6.8 (br, 1H, NH, exchangeable with D_2O), 7.47-7.26 (m, 7H, Ar-H + 2NH, exchangeable with D_2O).

4b: 59%, m.p.130-31 ˚C, $C_{24}H_{30}N_8O_5$ (510.56), Found: C 56.39, H 5.98, N 21.88, required: C 56.56, H 5.92, N 21.95. IR (γ/cm^{-1}): 3280 (NH), 2232 (CN), 1749 (CO, ester), 1645 (CO, amide). ^{1}H-NMR (DMSO-d$_6$): 1.08-1.02 (d, 3H, γ-CH$_3$), 1.25 (s, 9H, 3xCH$_3$), 2.14 (s, 3H, C-CH$_3$), 3.08 (s, 3H, N-CH$_3$), 4.22-4.18 (m, 1H, β-CH), 4.82-4.86 (d, 1H, α-CH), 5.56-5.60 (br, 1H, OH), 6.50-6.40 (br, 1H, NH, exchangeable with D$_2$O), 7.50-6.90 (m, Ar-H + 3xNH, exchangeable with D$_2$O).

4c: 54%, m.p.160-2 ˚C, $C_{29}H_{32}N_8O_4$ (556.63), Found: C 62.49, H 5.75, N 20.00, required: C 62.58, H 5.80, N 20.13. IR (γ/cm^{-1}): 3365 (NH), 2225 (CN), 1742 (CO, ester), 1641 (CO, amide). ^{1}H-NMR (DMSO-d$_6$): 1.31 (s, 9H, 3xCH$_3$), 2.16 (s, 3H, C-CH$_3$), 2.80 (m, 1H, α-CH), 3.13 (s, 3H, N-CH$_3$), 3.3 (d, 2H, β-CH$_2$), 6.7 (br, 1H, NH, exchangeable with D$_2$O), 7.5-6.7 (m, 12H, 2xAr-H + 2NH, exchangeable with D$_2$O), 8.5 (br, 1H, NH exchangeable with D$_2$O).

4d: 45%, m.p.170-71, $C_{26}H_{30}N_{10}O_4$ (546.64), Found: C 56.99, H 5.47, N 25.56, required: C 57.13, H 5.53, N 25.63. IR (γ/cm^{-1}): 3315 (NH), 2224 (CN), 1737 (CO, ester), 1649 (CO, amide). ^{1}H-NMR (DMSO-d$_6$): 1.29 (s, 9H, 3xCH$_3$), 2.1 (s, 3H, C-CH$_3$), 2.99 (s, 3H, N-CH$_3$), 3.86 (d, 2H, β-CH$_2$), 4.71 (m, 1H, α-CH), 6.7 (br, 1H, NH, exchangeable with D$_2$O), 7.5-7.1 (m, 7H, Ph + 2NH exchangeable with D$_2$O), 7.89 (d, 1H. 4-H, his.), 8.22 (s, 1H, 2-H, his.), 8.9 (br, 1H, NH, exchangeable with D$_2$O).

1-Phenyl-2,3-dimethyl-4-[-3-5-substituted amino-4-carbomethoxy)-1*H*-pyrazolyl-amino]pyrazolin-5-one derivatives (4e-h):

General procedure:

To a cold solution of protected amino acid (0.0015 mol) and *N*-hydroxybenzotriazole (0.0015 mol) in tetrahydrofuran, compound (**3b**) (0.0015 mol) was added. DCC (0.0015 mole) in tetrahydrofuran was added portionwise, while stirring and the temperature was kept at 0-5 ˚C. The dicyclohexayl urea was filtered off and ethyl acetate was evaporated under vacuum, and the residue was triturated with acetonitrile to remove all urea formed. Acetonitrile was filtered off and dried under vacuum, and the residue was dissolved in ethyl acetate, washed with distilled water, NHCO$_3$ (5%), distilled water, hydrochloric acid (0.5N) and finally with distilled water and dried over anhydrous sodium sulphate. The solvent was evaporated and the obtained product was purified by preparative TLC to afford (**4e-h**).

4e: 50%, m.p.102-103 ˚C, $C_{26}H_{35}N_7O_6$ (541.60), Found: C 57.69, H 6.41, N 18.16, required: C 57.77, H 6.34, N 18.14. IR (γ/cm^{-1}): 3320 (NH), 1750 (CO, ester), 1640 (CO, amide). ^{1}H-NMR (DMSO-d$_6$): 0.92-0.80 (m, 6H, 2xCH$_3$), 1.38 (s, 9H, 3xCH$_3$), 2.33 (s, 3H, C-CH$_3$), 2.29-2.36 (m, 1H, β-CH), 4.80 (m, 1H, α-CH), 3.1 (s, 3H-N-CH$_3$), 3.71 (s, 3H, OCH$_3$), 6.73 (br, 1H, NH, exchangeable with D$_2$O), 7.5-7.10 (m, 7H, Ar-H + 2NH, exchangeable with D$_2$O), 8.60 (rb, 1H, NH, exchangeable with D$_2$O). Ms : m/z (%) 542.3 (M^+, 20%).

4f: 54%, m.p.112-114, $C_{25}H_{33}N_7O_7$ (543.58), Found: C 55.18, H 6.08, N 18.00, required: C 55.24, H 6.12, N 18.04. IR (γ/cm^{-1}): 3370 (NH), 1747 (CO, ester), 1643 (CO, amide). ^{1}H-NMR (DMSO-d$_6$): 1.25 (s, 9H, 3xCH$_3$), 2.33 (s, 3H, C-CH$_3$), 3.00 (s, 3H-N-CH$_3$), 3.81 (s, 3H, OCH$_3$), 4.28-4.22 (m, 1H, β-CH), 4.86-4.82 (m, 1H, α-CH), 5.60-5.57 (br, 1H, OH, exchangeable with D$_2$O), 6.51-6.47 (br, 1H, NH, exchangeable with D$_2$O), 7.13 (br, 1H, NH, exchangeable with D$_2$O), 7.61-7.32 (m, 8H, Ar-H + 2NH, exchangeable with D$_2$O), 8.5 (br, 1H, NH exchangeable with D$_2$O).

4g: 56%, m.p.128-30 ˚C, $C_{30}H_{35}N_7O_6$ (589.65), Found: C 61.10, H 5.87, N 16.60, required: C 61.11, H 5.98, N 16.63. IR (γ/cm^{-1}): 3240 (NH), 1750 (CO, ester), 1690 (CO, amide). ^{1}H-NMR (DMSO-d$_6$): 1.38 (s, 9H, 3xCH$_3$), 2.35 (s, 3H, C-CH$_3$), 3.07 (s, 3H-N-CH$_3$), 3.88 (s, 3H, OCH$_3$), 3.30 (d, 2H, CH$_2$-Ph), 5.1 (q, 1H, α-CH), 6.8 (br, 1H, NH, exchangeable with D$_2$O), 7.56-7.21 (m, 12H, Ar-H + 2xNH, exchangeable with D$_2$O), 8.8 (br, 1H, NH exchangeable with D$_2$O).

4h: 51%, m.p.137-39, $C_{27}H_{33}N_9O_6$ (579.63), Found: C 55.87, H 7.68, N 21.73, required: C 55.95, H 5.74, N 21.75. IR (γ/cm^{-1}): 3230 (NH), 1729 (CO, ester), 1630 (CO, amide). ^{1}H-NMR (DMSO-d$_6$): 1.23 (s, 9H, 3xCH$_3$), 2.2 (s, 3H, C-CH$_3$), 3.10 (s, 3H, N-CH$_3$), 3.71 (s, 3H, OCH$_3$), 3.91 (d, 2H, β-CH$_2$), 4.81(m, 1H, α-CH), 6.80 (br, 1H, NH, exchangeable with D$_2$O), 7.6-7.1 (m, 7H, Ar-H + NH, exchangeable with D$_2$O), 8.19 (s, 1H, 2-H, his.), 7.83 (d, 1H, 4-H, his.), 9.01 (br, 1H, NH, exchangeable with D$_2$O).

Cleavage of *tert*-butyloxycarbonyl (Boc) blocking group: Formation of (5a-h).

General procedure

Boc-group was removed by dissolving compounds (**4a-h**) in CH_2Cl_2/TFA (1:1) and keeping for 20 min. at room temperature (TLC control). The solution was concentrated under vaccum. Deprotected compounds were precipitated by ether, filtered off and washed with ether several times to afford (**5a-h**).

5a: 49%, m.p.140-45 ˚C, $C_{20}H_{24}N_8O_2$ (408.45), Found: C 58.88, H 5.73, N 27.41, required: C 58.96, H 5.69, N 27.50. IR (γ/cm^{-1}): 3360 (NH_2), 2245 (CN), 1634 (CO, amide). ^{1}H-NMR (DMSO-d_6): 0.81 (d, 3H, γ-CH_3), 0.86 (d, 3H, γ-CH_3), 2.25 (s, 3H, C-CH_3), 2.41 (m, 1H, β-CH), 3.14 (s, 3H, N-CH_3), 5.21 (m, 1H, α-CH), 6.9 (br, 1H, NH, exchangeable with D_2O), 7.31-7.61 (m, 7H, Ar-H + 2 NH, exchangeable with D_2O), 8.6-8.3 (br, 2H, NH_2, exchangeable with D_2O).

5b: 50%, m.p.181-83 ˚C, $C_{19}H_{22}N_8O_3$ (410.44), Found: C 55.57, H 5.37, N 27.22, required: C 55.60, H 5.40, N 27.30. IR (γ/cm^{-1}): 3340 (NH_2), 2254 (CN), 1621 (CO, amide). ^{1}H-NMR (DMSO-d_6): 1.29-1.25 (d, 3H, γ-CH_3), 2.10 (s, 3H, C-CH_3), 3.06 (s, 3H, N-CH_3), 4.31-4.26 (m, 1H, β-CH), 4.56-4.53 (d, 1H, α-CH), 4.61-4.68 (br, 1H, OH, exchangeable with D_2O), 7.08 (br, 1H, NH, exchangeable with D_2O), 7.5-7.15 (m, 7H, Ar-H + NH, exchangeable with D_2O), 8.21-8.0 (br, 2H, NH_2, exchangeable with D_2O).

5c: 47%, m.p.133-5 ˚C, $C_{24}H_{24}N_8O_2$ (456.51), Found: C 63.00, H 5.28, N 24.47, required: C 63.15, H 5.30, N 24.54. IR (γ/cm^{-1}): 3348 (NH_2), 2313 (CN), 1637 (CO, amide). ^{1}H-NMR (DMSO-d_6): 2.21 (s, 3H, C-CH_3), 2.79 (m, 1H, α-CH_3), 3.01 (s, 3H, N-CH_3), 3.28 (d, 2H, β-CH_2), 6.79 (br, 1H, NH, exchangeable with D_2O), 7.5-7.1 (m, 11H, 2Ar-H + NH, exchangeable with D_2O), 8.7-8.51 (br, 2H, NH_2, exchangeable with D_2O).

5d: 40%, m.p.197-8 ˚C, $C_{21}H_{22}N_{10}O_2$ (446.43),Found: C 56.42, H 4.83, N 31.34, required: C 56.50, H 4.97, N 31.38. IR (γ/cm^{-1}): 3399 (NH_2), 2254 (CN), 1628 (CO, amide). ^{1}H-NMR (DMSO-d_6): 2.2 (s, 3H, C-CH_3), 3.01 (s, 3H, N-CH_3), 4.01 (d, 2H, β-CH_2), 4.82 (m, 1H, α-CH), 6.8 (br, 1H, NH, exchangeable with D_2O), 7.6-7.21 (m, Ar-H + 2NH, exchangeable with D_2O), 8.01 (d, 1H, 4-H, his.), 8.32 (s, 1H, 2-H, his.), 9.21 (br, 3H, NH + NH_2, exchangeable with D_2O).

5e: 52%, m.p.122-23 ˚C, $C_{21}H_{27}N_7O_4$ (441.40), Found: C 57.18, H 6.03, N 22.18, required: C 57.27, H 5.95, N 22.26. IR (γ/cm^{-1}): 3355 (NH_2), 1760, (CO, ester), 1633 (CO, amide). ^{1}H-NMR (DMSO-d$_6$): 1.01-0.94 (m, 6H, 2xCH_3), 2.29 (s, 3H, C-CH_3), 2.30-2.22 (m, 1H, β-CH), 3.31 (s, 3H, N-CH_3), 3.85 (s, 3H, OCH_3), 4.73 (m, 1H, α-CH), 6.73 ((br, 1H, NH, exchangeable with D_2O), 7.63-7.21 (m, 5H, Ar-H + NH, exchangeable with D_2O), 8.7 (br, 2H, NH_2, exchangeable with D_2O).

5f: 52%, oily, $C_{20}H_{25}N_7O_5$ (443.38), Found: C 54.09, H 5.74, N 22.06, required: C 54.18, H 5.68, N 22.11. IR (γ/cm^{-1}): 3349 (NH_2), 1750, (CO, ester), 1699 (CO, amide). ^{1}H-NMR (DMSO-d$_6$): 2.24 (s, 3H, C-CH_3), 3.02 (s, 3H, N-CH_3), 3.82 (s, 3H, OCH_3), 4.17-4.00 (m, 1H, β-CH), 4.73-4.65 (m, 1H, α-CH), 5.56-5.49 (br, 1H, OH, exchangeable with D_2O), 6.73-6.66 (br, 1H, NH exchangeable with D_2O), 7.2 (br, 1H, NH, exchangeable with D_2O), 7.55-7.27 (m, 6H, Ar-H + NH, exchangeable with D_2O), 8.71 (br, 2H, NH_2, exchangeable with D_2O).

5g: 45%, oily, $C_{25}H_{27}N_7O_4$ (489.45), Found: C 61.28, H 5.48, N 20.10, required: C 61.35, H 5.56, N 20.03. IR (γ/cm^{-1}): 3401 (NH_2), 1729, (CO, ester), 1629 (CO, amide). ^{1}H-NMR (DMSO-d$_6$): 2.32 (s, 3H, C-CH_3), 3.12 (s, 3H, N-CH_3), 3.4 (d, 2H, CH_2-Ph), 3.76 (s, 3H, OCH_3), 5.2 (q, 1H, α-CH), 6.65 (br, 1H, NH, exchangeable with D_2O), 7.56-7.16 (m, 11H, 2Ar-H + NH, exchangeable with D_2O), 8.72 (br, 2H, NH_2, exchangeable with D_2O).

5h: 56%, m.p.117-19 ˚C, $C_{22}H_{25}N_9O_4$ (479.42), Found: C 55.18, H 5.28, N 26.27, required: C 55.12, H 5.26, N 26.29. IR (γ/cm^{-1}): 3399 (NH_2), 1735, (CO, ester), 1625 (CO, amide). ^{1}H-NMR (DMSO-d$_6$): 2.3 (s, 3H, C-CH_3), 3.2 (s, 3H, N-CH_3), 3.81 (s, 3H, OCH_3), 3.93 (d, 2H, β-CH_2), 4.93 (m, 1H, α-CH), 6.91 (br, 1H, NH, exchangeable with D_2O), 7.63-7.31 (m, 6H, Ar-H + NH, exchangeable with D_2O), 7.93 (d, 1H, 4-H, his.), 8.22 (s, 1H, 2-H, his.), 8.51 (br, 2H, NH_2, exchangeable with D_2O), 9.33 (br, 1H, NH, exchangeable with D_2O).

Formation of dipeptide (6):

Synthesis of (**6**) was done via the active ester method using DCC and *N*-hydroxy-succinimide (HoSu). The procedure as described in the preparation of compounds (**4a-d**),

using compound (**5c**) as starting material to couple with carbobenzoxy-L-leucine to afford compound (**6**).

6: 60%, m.p. oily, $C_{38}H_{41}N_9O_5$ (703.81), Found: C 64.94, H 5.78, N 17.83, required: C 64.85, H 5.87, N 17.91. IR (γ/cm^{-1}): 3220 (NH), 2230 (CN), 1735, (CO, ester), 1645 (CO, amide). ^{1}H-NMR ($CDCl_3$): 0.86 (m, 6H, 2xCH_3), 1.2-1.3 (m, 2H, CH_2), 2.22 (s, 3H, C-CH_3), 2.33 (m, 1H, CH), 3.14 (s, 3H, N-CH_3), 3.2 (t, 1H, CH), 3.35 (d, 2H, CH_2-Ph), 4.3 (m, 1H, CH-NH), 4.9 (m, 1H, CH-NH), 6.7 (br, 1H, NH, exchangeable with D_2O), 7.5-7.1 (m, 11H, Ar-H + NH, exchangeable with D_2O), 8.2 (br, 1H, NH, exchangeable with D_2O), 9.2 (br, 2H, 2NH, exchangeable with D_2O).

1-Phenyl-2,3-dimethyl-4-[3(5-thioamido)valin-4-(carbomethoxy)-1*H*-pyrazolyl-amino]pyrazolin-5-thion (7) :

A mixture of compound (**5e**) (0.001 mol) and (0.001 mol) of Lawesson's reagent in (6 ml) anhydrous benzene was refluxed for 3 hr. (TLC control). The reaction mixture after adding charcoal was kept cold (0 °C) overnight, then filtered off and the solvent was evaporated under reduced pressure. The oily residue was purified on preparative TLC affording compound (7) in 50% yield. IR (γ/cm^{-1}): 3440 (NH), 1735 (CO, ester), 1450 (C=S, thioamide). ^{1}H-NMR: 2.3 (s, 3H, C-CH_3), 3.11 (s, 3H, N-CH_3), 3.81 (s, 3H, OCH_3), 4.68-4.49 (m, 1H, β-CH), 5.21-5.13 (m, 1H, α-CH), 6.1-6.0 (br, 1H, OH, exchangeable with D_2O), 7.2-7.1 (br, 1H, NH, exchangeable with D_2O), 7.4 (br, 1H, NH, exchangeable with D_2O), 8.1-7.5 (m, 6H, Ar-H + NH, exchangeable with D_2O), 9.2 (br, 2H, NH_2, exchangeable with D_2O),

1-Phenyl-2,3-dimethyl-4-[-3-(5-methylpyrazolo[3,4-*d*]pyrimidin-3-one)-1*H*-pyrazolylamino]pyrazolin-5-one (8):

A mixture of (**3a**) (0.0015 mole) and 10 ml acetic anhydride was refluxed for 10 hr. The product was crystallized from acetic acid to afford (8) in 60% yield, m.p. > 300 °C. $C_{17}H_{17}N_7O_2$ (351.38), Found: C 58.00, H 4.73, N 27.8, required: C 58.11, H 4.88, N 27.90. ^{1}H-NMR (DMSO-d_6): 2.1 ((s, 3H, C-CH_3), 2.3 (s, 3H, C-CH_3), 3.1 (s, 3H, N-CH_3), 6.9 (br, 1H, NH, exchangeable with D_2O), 7.5-7.1 (m, 7H, Ar-H + 2NH, exchangeable with D_2O).

1-Phenyl-2,3-dimethyl-4-[3-(4-*N*-acetyl-3carbomethoxy-5-methylpyrazolo[1,5-*a*]-pyrimidin-7-one)pyrazolylamino]pyrazolin-5-one (9):

A mixture of (**3b**) (0.0015 mole) and 10 ml acetic anhydride was refluxed for 10 hrs. The product was crystallized from acetic acid to afford compound (9) in 65% yield, m.p. 295 °C. $C_{22}H_{22}N_6O_5$ (450.45), Found: C 58.67, H 4.95, N 18.68, required: C 58.65, H 4.93, N 18.66. IR (γ/cm^{-1}): 1730 (C=O, ester), 1675 (N-CO), 1615 (N-CO-CH_3). ^{1}H-NMR (DMSO-d$_6$): 2.3 (s, 3H, C-CH_3), 2.4 (s, 3H, C-CH3), 3.1 (s, 3H, N-CH_3), 3.41 (s, 3H, NCO-CH3), 3.8 (s, 3H, $COOCH_3$), 7.1 (s, 1H, cyclic CH), 7.7-7.29 (m, 6H, Ar-H + NH, exchangeable with D_2O). MS, m/z (%): 451 (10, M^+), 399 (5), 384 (100), 325 (10), 310 (20), 264 (15), 77 (55).

1-Phenyl-2,3-dimethyl-4-[3-(4*H*-3-carbomethoxy-5-methylpyrazolo[1,5-*a*]-pyrimidin-7-one)pyrazolylamino]pyrazolin-5-one (10B):

A mixture of (**3b**) (0.0015 mol) and ethyl acetoacetate (10 ml) in 10 ml acetic acid was refluxed for 10 hrs. The product was crystallized from dioxane to afford compound **10b** in 70% yield, m.p. >. 300 °C. $C_{20}H_{20}N_6O_4$ (408.42), Found:C 58.72, H 4.83, N 20.49, required : C 58.82, H 4.94, N 20.58. IR (γ/cm^{-1}): 1743 (C=O, ester), 1670 (C=O). ^{1}H-NMR (DMSO-d$_6$): 2.2 (s, 3H, C-CH_3), 3.1 (s, 3H, N-CH_3), 3.75 (s, 3H, $COOCH_3$), 7.0 (s, 1H. cyclic CH), 7.7-7.2 (m, 6H, Ar-H + NH, exchangeable with D_2O), 8.1 (br, 1H, NH, exchangeable with D_2O). MS: m/z (%): 409 (100, M^+).

1-Phenyl-2,3-dimethyl-4-[3-(5-arylidinemethyline-4-cyano)-1*H*-pyrazolyl-amino]pyrazolin-5-one (11a,b):

A mixture of (**3a**) (0.0015 mol) and 4-bromobenzaldehyde or 5-methyl furfural (0.0015 mol) in acetic acid/dioxane (1:1) was refluxed for 12 hrs. The product was crystallized from acetic acid to afford (**11a**) and (**11b**), respectively.

11a: 70%, m.p. 275 °C, $C_{22}H_{18}N_7OBr$ (476.34), Found: C 55.29, H 3.70, N 20.39, required: C 55.47, H 3.81, N 20.58. IR (γ/cm^{-1}): 2240 (CN). ^{1}H-NMR (DMSO-d$_6$): 2.1 (s, 3H, C-CH_3), 3.1 (s, 3H, N-CH_3), 6.95 (br, 1H, NH, exchangeable with D_2O), 7.77 (d, 2H, Ph-Br-p), 7.96 (d, 2H, Ph-Br-p), 7.5-7.2 (m, 6H, Ar-H + NH, exchangeable with D_2O), 8.9 (s, 1H, N=CH). MS, m/z (%) : 477 (20, M^+).

11b: 55%, m.p. 260 °C, $C_{21}H_{19}N_7O_2$ (401.43), Found: C 62.57, H 4.48, N 24.46, required: C 62.83, H 4.77, N 24.42. IR (γ/cm^{-1}): 2260 (CN). ^{1}H-NMR: did not dissolve in deuterated solvent. MS, m/z (%): 402 (25, M^+).

1-Phenyl-2,3-dimethyl-4-[3-(4-carbhydrazide-5-substituted–5-methylpyrazolo[1,5-*a*] pyrimidin-7-one)pyrazolylamino]pyrazolin-5-one (14a,b):

A mixture of **9** or **10** (0.0015 mol) and hydrazine hydrate (0.002 mol) in ethanol was refluxed for 6 hrs. (TLC control). A white precipitate formed, filtered off and crystallized from DMF to afford **14a** (60%) and **14b** (70%) yield, respectively.

14a: m.p. > 300 °C, $C_{19}H_{20}N_8O_3$ (408.27), Found: C 55.91, H 4.97, N 27.47, required: C 55.89, H 4.95, N 27.45. ^{1}H-NMR: 2.2 (s, 3H, C-CH_3), 3.1 (s, 3H, N-CH_3), 3.6 (s, 3H, CH_3), 5.6 (br, 2H, NH_2, exchangeable with D_2O), 7.6-7.2 (m, 8H, Ar-H + cyclic CH + 2NH, exchangeable with D_2O), 9.5 (br, 1H, NH, exchangeable with D_2O).

14b: m.p. > 300 °C, $C_{21}H_{22}N_8O_4$ (450.51), Found: C 56.00, H 4.95, N 24.90, required: C 55.98, H 4.93, N 24.88. ^{1}H-NMR: 2.1 (s, 3H, C-CH_3), 3.1 (s, 3H, CH_3), 3.62 (s, 3H, CH_3), 3.80 (s, 3H, $COCH_3$), 6.2 (br, 2H, NH_2 exchangeable with D2O), 7.7-7.3 (m, 7H, Ar-H + cyclic CH, NH exchangeable with D_2O), 9.9 (br, 1H, NH, exchangeable with D_2O).

1-Phenyl-2,3-dimethyl-4-[3-(4-(2-methyl-1,3,4-oxadiazol)-4-acetyl-1*H*-5-methyl-pyrazolo[1,5-*a*]pyrimidin-7-one)pyrazolylamino]pyrazolin-5-one (13):

Method A :

A mixture of (**14a**) (0.0015 mol) and (10 ml) acetic anhydride was refluxed for 10 hrs. A white precipitate was formed, and recrystallized from acetic acid to afford (13) in 55% yield, m.p. > 300 °C. $C_{23}H_{22}N_8O_4$ (474.48), Found: C 58.15, H 4.51, N 23.64, required: C 58.22, H 4.67, N 23.62. ^{1}H-NMR (DMSO-d_6): 2.3 (s, 3H, C-CH_3), 2.4 (s, 3H, C-CH_3), 3.2 (s, 3H, N-CH_3), 3.4 (s, 3H, CH_3), 3.8 (s, 3H, $COCH_3$), 7.1 (s, 1H, cyclic CH), 7.5 -7.2 (m, 6H, Ar-H + CH + NH, exchangeable with D_2O). MS, m/z (%): 385 (M^+ -3xCH_3 & $COCH_3$) (90), 342 (20), 310 (60), 83 (20).

Method B.

A mixture of **9** (0.0015 mol) and acetohydrazide (0.0015 mol) in ethyl alcohol was refluxed for 10 hrs. A white precipitate was formed, crystallized from acetic acid to afford **13** (70%).

1-Phenyl-2,3-dimethyl-4-[3-(4-(3-*p*-bromophenylhydrazono)-4-*N*-acetyl-5-methyl-pyrazolo[1,5-*a*]pyrimidin-7-one)pyrazolylamino]pyrazolin-5-one (15):

A mixture of **13** (0.0015 mole) and *p*-bromobenzaldehyde in dioxane was refluxed for 8 hrs. (TLC control). The product was crystallized from dioxane to afford (15) in 49% yield, m.p. > 300 °C. $C_{28}H_{25}N_8O_4Br$ (617.46), Found: C54.31, H4.00, N 18.09, required: C 54.47, H 4.08, N 18.15. ^{1}H-NMR (DMSO-d_6): 2.1 (s, 3H, C-CH_3), 3.2 (s, 3H, N-CH_3), 3.6 (s, 3H, CH_3), 3.7 (s, 3H, $COCH_3$), 7.1 (s, 1H, cyclic CH), 7.6-7.2 (m, 6H, Ar-H + NH, exchangeable with D_2O), 8.5 (s, 1H, N=CH), 9.2 (s, 1H, NH, exchangeable with D_2O). MS, m/z (%) : 481 (M^+ - CHPhBr) (20), 342 (80) 310 (40).

4-Amino-3-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)amino]-5-sulfanyl-1,7-dihydro-6*H*-pyrazolo[3,4-*b*]pyridin-6-one (16):

A solution of DCC (0.0015 mol) in dimethylformamide was added portionwise to a stirred solution of thioglycollic acid (0.0015 mole) and N-hydroxysuccinimide (HoSu) in 10 ml dimethylformamide at (0-5 °C). A cold solution of **3a** (0.0015 mol) in 10 ml dimethylformamide was added at the same temperature with adjustment of the pH to 8. The mixture was stirred for 3 hrs at 0 °C overnight, and at room temperature for 48 hrs. Drops of acetic acid were added. The precipitate, dicyclohexayl urea was filtered off. The reaction mixture was then poured over ice, stirred well and the precipitate formed was filtered off and washed several times with water and sodium carbonate. The solid crystallized from ethanol to afford (16) in 45% yield, m.p. 255 °C. $C_{17}H_{17}N_7O_2S$ (383.44), Found: C 53.13, H 4.34, N 25.31, required: C 53.25, H 4.47, N 25.57. IR (γ/cm^{-1}): 3450 (NH_2), 2600 (SH), 1630 (C=O, amide). ^{1}H-NMR (DMSO-d_6): 2.1 (s, 3H, C-CH_3), 3.1 (s, 3H, N-CH_3), 3.2 (s, 1H, SH, exchangeable with D_2O), 4.5 (br, 2H, NH_2, exchangeable with D_2O), 7.4 -7.1 (m, 6H, Ar-NH, exchangeable with D_2O), 6.8 (br, 1H, NH, exchangeable with D_2O), 8.6 (s, 1H, NH, exchangeable with D_2O). MS, m/z (%) : 367 (2)(M^+-NH_3), 310 (50), 249 (10), 189 (40), 56 (100).

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Povzetek

Reakcija derivatov ketena (**1a,b**) z 4-amino-1-fenil-2,3-dimetil-pirazolin-5-onom vodi do spojin (**2a,b**), ki reagirajo s hidrazinom in se pretvorijo v 1-fenil-2,3-dimetil-4-[3-(5-amino-4-substituran)-1*H*-pirazolilamino]pirazolin-5-on (3a,b).

Spojine (**3a,b**) dajo z Boc. amino kislinskimi derivati, acetanhidridom, etil acetoacetatom in aldehidi spojine (**4a-h**), (**5a-h**), (**6-10**), (**11a,b**). Spojini (**9**) ali (**10**) sta pri reakciji z act ohidrazidom oziroma hidrazini, s sledečo obdelavo s *p*-bromobenzaldehidom dali spojine (**13**), (**14a,b**) in (**15**). Spojina (**3a**) smo s tioglikolno kislino pretvorili v derivat pirazolopiridina (**16**).