

Scientific paper

Synthesis and Cyclization Reactions with Pyrazolopyrimidinyl Keto-esters and their Enzymatic Activity

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Abstract

Ethyl 2,4-dioxo-4-(4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)butanoate, ethyl 5-(4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)-1H-pyrazole-3-carboxylate and their acid hydrazide derivatives have been prepared and reacted with hydrazines, *ortho*-phenylenediamine, triethyl orthoformate, carbon disulfide and thiosemicarbazides in order to obtain some new 5-substituted pyrazolopyrimidin-4-ones as pyrazolines, isoxazolines, imidazoles, pyrazolotriazines, thiadiazoles and triazoles. All newly prepared compounds revealed the potent effect on increasing reactivity of cellobiase. Structures of new compounds were established upon their elemental analysis, IR, ¹H NMR and mass fragmentation spectra.

Keywords: Pyrazolines, isoxazolines, imidazoles, pyrazolotriazines, thiadiazoles and triazoles

1. Introduction

In connection with previous studies of the chemistry of substituted pyrazolopyrimidin-4-ones,^{1–4} this work deals with the synthesis of new pyrazolopyrimidinones substituted at position 5 with pyrazolyl, isoxazolyl, triazinyl and thiadiazolyl moieties. This stems from the recent notable biological applications of pyrazolopyrimidinones,^{5–7} pyrazoles,^{8,9} isoxazoles,^{10,11} triazines,^{12,13} and thiadiazoles.^{14–17} This encouraged us to prepare new heterocycles containing pyrazolopyrimidinone skeleton loaded with the latter substrates with the aim to improve the biological activity of pyrazolopyrimidine α,γ -diketoesters.

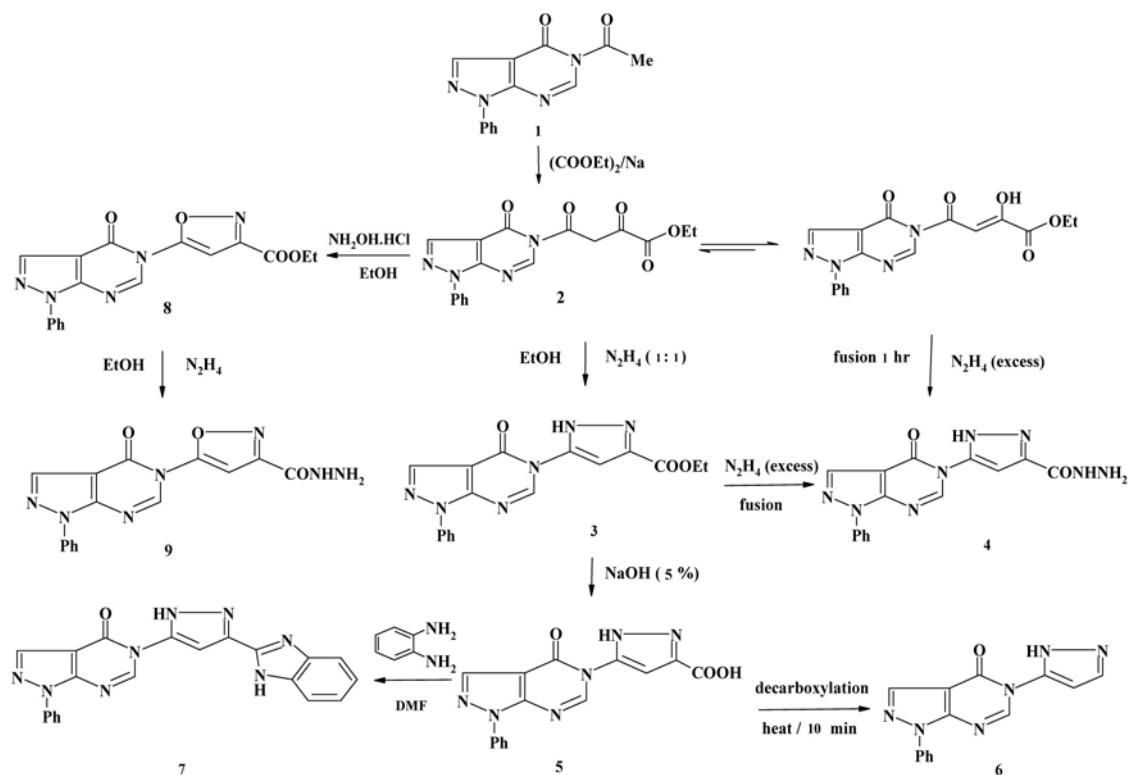
2. Results and Discussion

Due to the considerable chemical reactivity of pyrazolopyrimidine α,γ -diketoesters, ethyl 5-(4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)-1H-pyrazole-3-carboxylate (**3**) was synthesized (Scheme 1) and used as starting material to obtain some new 5-substituted pyrazolopyrimidinone derivatives.

The structure of α,γ -diketoester **2** was inferred on the basis of its spectral and analytical data. IR spectrum of

the ester **2** revealed the presence of carbonyl ester at its characteristic wave number 1725 cm⁻¹ in addition to the vibrational bands at 1660, 1650 and 1630 cm⁻¹ (α -C=O, γ -C=O and C=O_{pyrimidinone}, respectively). ¹H NMR spectrum of compound **2** showed signals at δ 1.25 (t) and 4.3 (q) specific for ethoxy group and at δ 4.1 specific for COCH₂CO. The mass fragmentation pattern of the ester **2** revealed molecular ion peak at m/z 354 (2%) and the base peak at m/z 281 corresponding to [C₁₄H₉N₄O₃]⁺.

For the purpose of obtaining various 5-substituted pyrazolopyrimidinones **2**, reaction with some *N*-nucleophiles, such as hydrazine, phenylhydrazine and hydroxylamine was checked at various molar ratio and conditions. Thus, when **2** reacted with hydrazine hydrate at the molar ratio 1:1 in boiling ethanol, ethyl 5-(4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)-1H-pyrazole-3-carboxylate (**3**) was formed (Scheme 1). On the other hand, using excess amount of hydrazine under fusion condition resulted in the acid hydrazide **4**, which was also obtained by the hydrazinolysis of **3** using excess of hydrazine. Elemental analyses and spectral data of compounds **3** and **4** are in good accordance with the suggested structures. IR spectrum of **3** showed the disappearance of the vibrational bands specific for α - and γ -carbonyl groups and the presence of absorption bands specific for the ester group at position 3 of the pyrazole. On the other



Scheme 1

hand, IR spectrum of **4** revealed bands at 3420 and 3300 cm^{-1} specific for NH_2 group and its ^1H NMR spectrum showed signal at δ 3.4 specific for NH_2 group of the acid hydrazide.

Hydrolysis of the ester **3**, using aqueous solution of sodium hydroxide furnished the carboxylic acid derivative

5, which gave positive acidity test. When compound **5** was heated above its melting point in the absence of a solvent a decomposition product was obtained, which was characterized by its analytical and spectral data and was deduced to be 1-phenyl-5-(1*H*-pyrazol-5-yl)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (**6**).

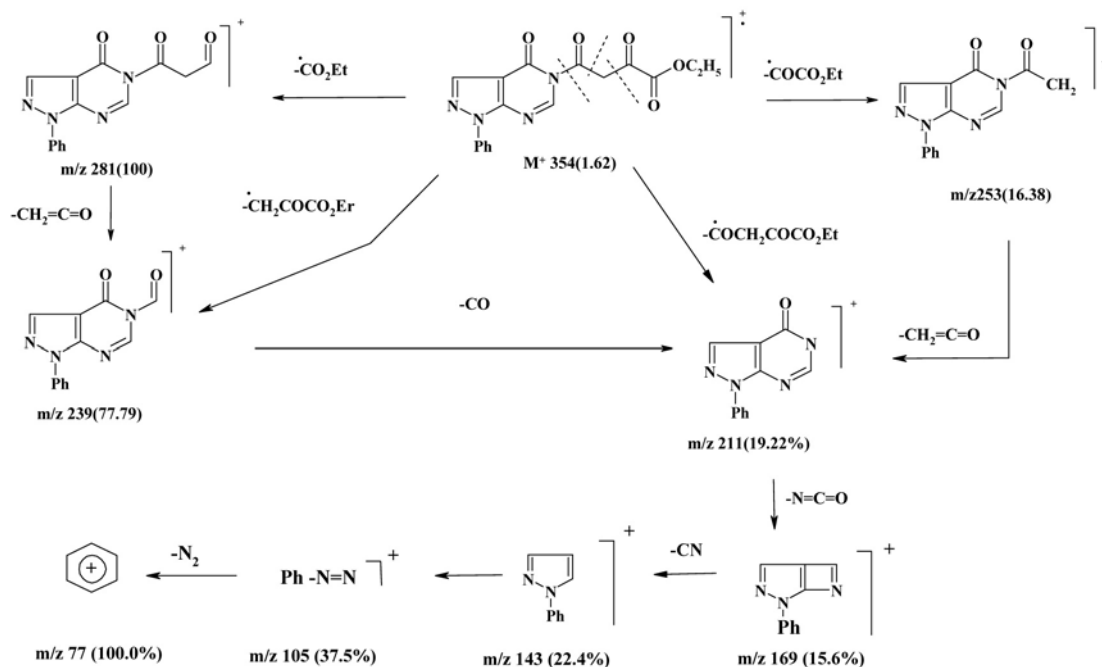
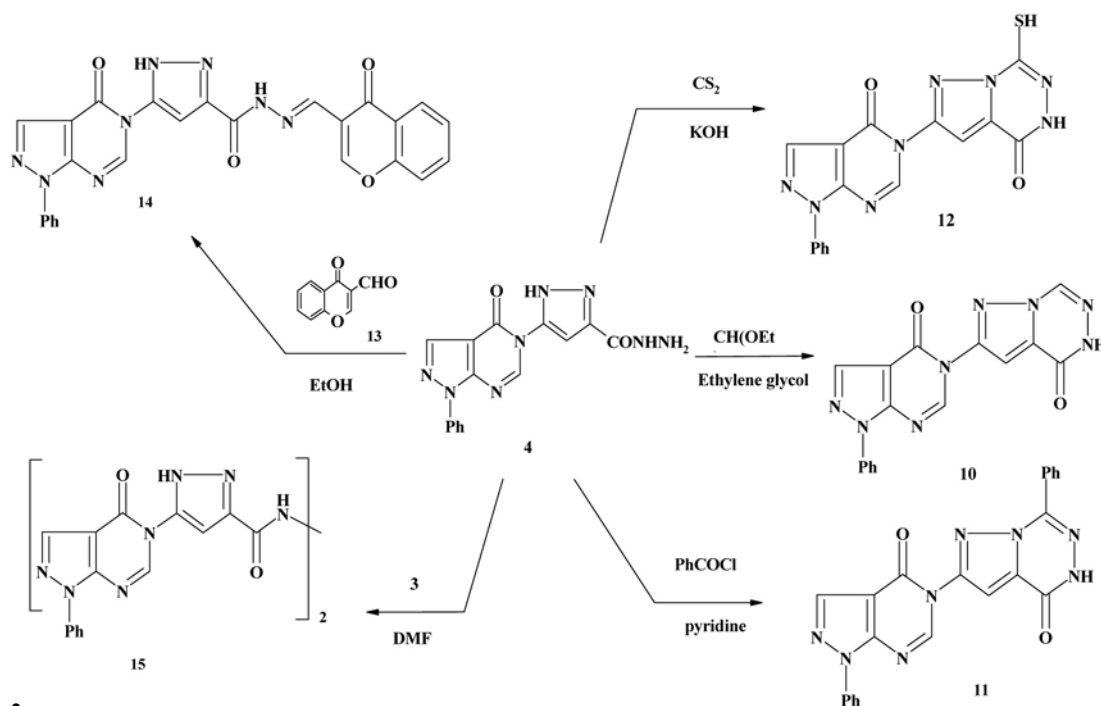


Chart 1. Fragmentation pattern of compound 2

Additional support for the structure of **3** was achieved by its reaction with *ortho*-phenylenediamine, where benzoimidazole derivative **7** was obtained.

In continuation of the study devoted to the investigation of the chemical reactivity of pyrazolopyrimidinone derivatives, the ketoester **2** was reacted with hydroxylammonium chloride in boiling ethanol to give isoxazole derivative **8**. The hydrazinolysis of the latter product, using the excess amount of hydrazine hydrate led to the formation of acid hydrazide **9**. The structures of both compounds **8** and **9** met satisfactory elemental analyses and spectral data.

For the purpose of obtaining various pyrazolotriazines attached directly to pyrazolopyrimidinone at position 5, the acid hydrazide **4** was reacted with some selected reagents. Thus, **4** was treated with triethyl orthoformate in ethylene glycol to give the pyrazolotriazine derivative **10** (Scheme 2).



Scheme 2

When the acid hydrazide **4** was allowed to react with benzoyl chloride in dry pyridine, cyclocondensation product **11** was obtained. The IR and ^1H NMR spectra of compounds **10** and **11** showed the inclusion of both amino groups due to the acid hydrazide along with NH group due to the pyrazole ring system in the cyclization process.

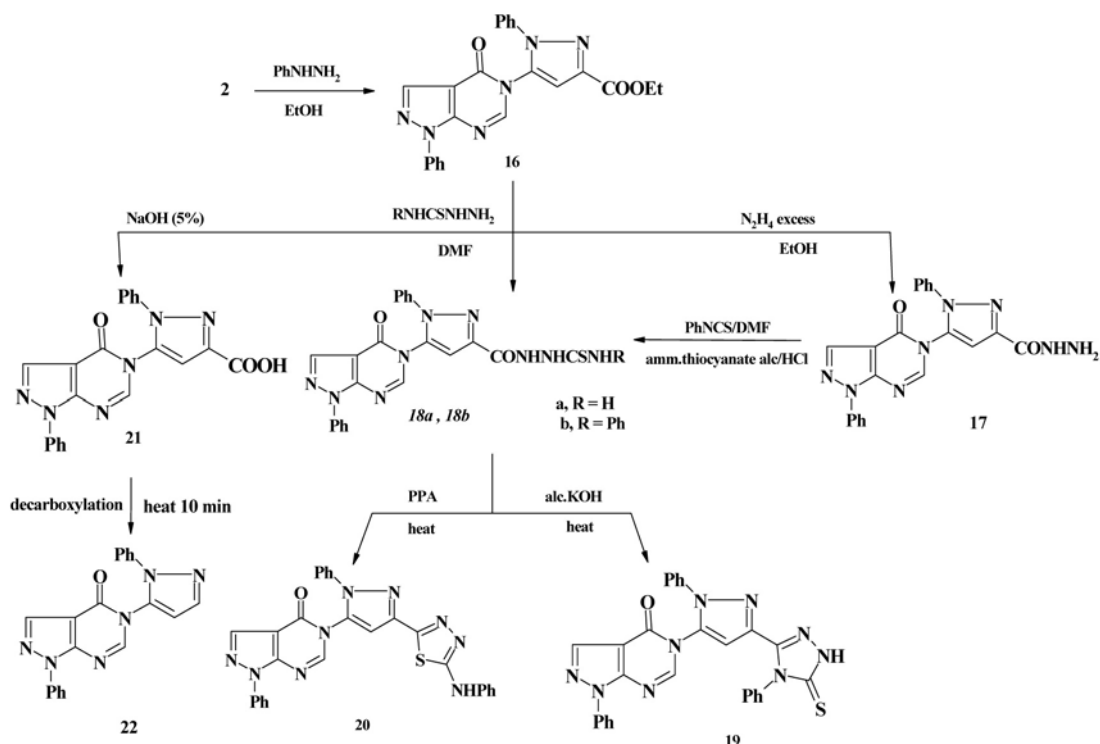
For obtaining another derivative of pyrazolopyrimidinone bearing pyrazolotriazine moiety, the reaction of the acid hydrazide **4** with carbon disulfide in the presence of alcoholic potassium hydroxide was investigated. Product was the desired pyrazolotriazine derivative **12**, IR spectrum of which revealed the presence of absorption band at 2660 cm^{-1} specific for SH group.

Treatment of the acid hydrazide **4** with equimolar amount of 4-oxo-4*H*-1-benzopyran-3-carboxaldehyde (**13**) in boiling ethanol afforded the corresponding hydrazone **14**, while the reaction of the compound **4** with the ester **3** led to the formation of the interesting bis(pyrazolopyrimidinylpyrazole)hydrazide **15**. IR spectra of the hydrazone **14** and the hydrazide **15** showed characteristic absence of the vibrational bands specific for the NH_2 group. Also elemental microanalyses of these compounds proved their proposed formulas.

Cyclocondensation between the diketoester **2** and phenylhydrazine was performed in ethanol to produce ethyl 5-(4-oxo-1-phenyl-1,4-dihydro-5*H*-pyrazolo[3,4-*d*]pyrimidin-5-yl)-1-phenyl-1*H*-pyrazole-3-carboxylate (**16**) (Scheme 3), which is considered as an appropriate precursor for synthesizing target compounds. Thus, the reaction of **16** with excess hydrazine hydrate gave the corresponding acid hydrazide **17**, while the condensation

reaction between the ester **16** and 4-substituted thiosemicarbazides afforded the corresponding pyrazole-3-carbonyl thiosemicarbazides **18a,b**. The same products were obtained when the acid hydrazide **17** was reacted with ammonium thiocyanate in boiling ethanol in the presence of hydrochloric acid and/or phenylisothiocyanate in DMF, respectively.

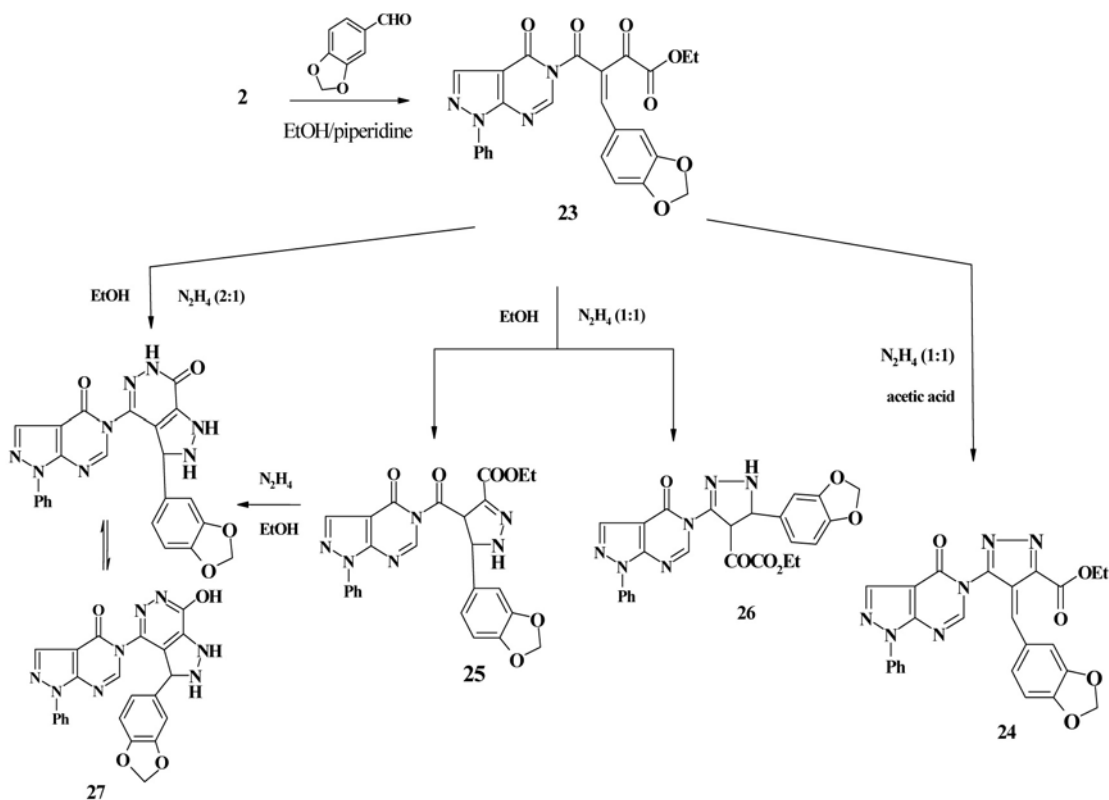
When compound **18b** was treated with alcoholic potassium hydroxide cyclization took place. In this reaction carbonylthiosemicarbazide side chain underwent intramolecular cyclocondensation and gave 1-phenyl-5-[1-phenyl-3-(4-phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)-1*H*-pyrazol-5-yl]-1,5-dihydro-



Scheme 3

4H-pyrazolo[3,4-d]pyrimidin-4-one (**19**). ^1H NMR spectrum of **19** revealed the presence of skeletal NH at δ 10.5 ppm, fortifying the proposed cyclization to triazole system. On the other hand, treatment of **18b** with polyphosphoric acid (PPA), furnished 5-[3-(5-phenylamino-1,3,4-thiadiazol-2-yl)-1-phenyl-1H-pyrazol-5-yl]-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (**20**).

phosphoric acid (PPA), furnished 5-[3-(5-phenylamino-1,3,4-thiadiazol-2-yl)-1-phenyl-1H-pyrazol-5-yl]-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (**20**).



Scheme 4

Such cyclization reaction found support in the spectral data which proved the elimination of a molecule of water besides the presence of a signal for NH at the H-aromatic zone due to phenylamino group.

The reactivity of the ester group of the pyrazole derivative **16** towards basic hydrolysis was also studied. Thus, hydrolysis of the ester **16** led to the carboxylic acid derivative **21**, which gave positive acidity test. When the acid **21** was subjected to decarboxylation reaction 1-phenyl-5-(1-phenyl-1*H*-pyrazol-5-yl)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (**22**) was formed. The elemental analysis of compound **22** is in good accordance with calculated values and IR spectrum shows the disappearance of the characteristic bands for carboxylic group.

Comparative study of the reactivity of α,β -unsaturated carbonyl group against ketoester group, when present in one molecular frame, has been carried out; thus, ethyl 4-(1,3-benzodioxol-5-yl)-2-oxo-3-[(4-oxo-1-phenyl-1,4-dihydro-5*H*-pyrazolo[3,4-*d*]pyrimidin-5-yl)carbonyl]-3-butenate (**23**) (Scheme 4) was synthesized by the action of piperonal on **2** in the presence of piperidine as a catalyst. ¹H NMR spectrum of the butenoic acid ester **23** showed distinctive chemical shifts at δ 5.55 (s) due to OCH₂O and δ 6.62 (s) due to an olefinic CH which revealed that condensation of piperonal took place at the active β -methylene of the diketester.

On treatment of compound **23** with hydrazine at the molar ratio 1:1 in glacial acetic acid, condensation reaction took place to give ethyl 4-(1,3-benzodioxol-5-ylmethylene)-5-(4-oxo-1-phenyl-1,4-dihydro-5*H*-pyrazolo[3,4-*d*]pyrimidin-5-yl)-4*H*-pyrazole-3-carboxylate (**24**). IR spectrum of compound **24** showed the disappearance of the vibrational bands specific for α - and γ -carbonyl groups and the presence of absorption bands due to the ester group at the position 3 of the pyrazole. ¹H NMR spectrum of the ester **24** gave much more information about the structure of this compound, showing peaks at δ 1.25 (t) and 4.14 (q) specific for ethoxy indicating that ester group is not participating in the cyclocondensation.

Surprisingly, on repeating the latter reaction at the same ratio but in ethanol instead of acetic acid the corresponding ethyl 5-(1,3-benzodioxol-5-yl)-4-[(4-oxo-1-phenyl-1,4-dihydro-5*H*-pyrazolo[3,4-*d*]pyrimidin-5-yl)carbonyl]-4,5-dihydro-1*H*-pyrazole-3-carboxylate (**25**) was obtained, however neither **24** nor **26** were formed. The IR spectrum of the product revealed the presence of C=O and COOEt characterized by vibrational absorption at 1670 cm⁻¹ for keto C=O and 1730 cm⁻¹ for C=O_{ester} indicating that the ester group was still present and not involved in the cyclization process.

Beside the analytical and spectral evidences for the structure of **25** a good support for its chemical structure was achieved by the reaction of **25** with another mole of hydrazine hydrate in ethanol to give 5-[3-(1,3-benzodioxol-5-yl)-7-hydroxy-2,3-dihydro-1*H*-pyrazolo[3,4-*d*]pyridazin-4-yl]-1-phenyl-1,5-dihydro-4*H*-pyrazolo[3,4-

d]pyrimidin-4-one (**27**). The evident formation of the latter product indicated that compound **25** must contain a free carbonyl group and an ethoxycarbonyl group, which are involved in the cyclization reaction to form the fused pyrazolopyridazine system. On the other hand, when compound **25** was condensed with hydrazine hydrate at the ratio 1:2 in ethanol, the product formed was found to be identical in every respect to the product that was obtained by the action of N₂H₄ on compound **25**. The formation of **27** by these two pathways is considered a good support for the structure of both **25** and **27**.

3. Cellobiase Activity Test

The effect of the newly prepared compounds on the activity of cellobiase, an enzyme produced by a thermotolerant fungus *Absidia corymbifera*, was investigated.¹⁸ The results showed (Table 1) that most of the tested compounds enhanced the effect of the enzyme in the production of glucose (ρ_{glucose} 1.37–3.25 $\mu\text{g cm}^{-3}$). The data obtained proved that compound **23** is the most active one (3.25 $\mu\text{g cm}^{-3}$), this may be due to the presence of α,γ -diketoester and α,β -unsaturated system in one molecular frame; this might also be supported by the amount of glucose produced by the effect of α,γ -diketoester derivative **2** (2.60 $\mu\text{g cm}^{-3}$). On the other hand, these results also showed that the relatively high values (2.10–2.35 $\mu\text{g cm}^{-3}$) may be due to the presence of pyrazolotriazines, substituted pyrazoles or triazoles and thiadiazoles bearing pyrazolopyrimidinone.

Generally all compounds activate enzyme, activity may be due to the presence of more than one heterocyclic ring and hetero atoms. The most active one was **23** whereas the least active was **7**. The compounds could be arranged in relation to their promoting effect in the following order: **23** > **24** > **25** > **27** > **2** > **20** > **13** > **21** > **11** > **12**–**18a** > **15**.

Table 1. Effect of new compounds on activity of cellobiase.^a

Compound	ρ (Glucose) $\mu\text{g cm}^{-3}$	Compound	ρ (Glucose) $\mu\text{g cm}^{-3}$
2	2.60	16	1.87
3	1.55	17	1.73
4	1.75	18	1.89
5	1.83	18a	2.00
6	2.10	18b	1.97
7	1.37	20	2.35
8	1.70	21	2.23
9	1.77	22	2.15
10	1.80	23	3.25
11	2.10	24	3.23
12	2.00	25	2.95
13	2.30	27	2.89
14	1.60	Blank ^a	0.59
15	1.99	Control ^b	0.28

^a Blank test using bidistilled water produced 0.592 $\mu\text{g cm}^{-3}$.

^b Using DMF (0.1 mL) without sample.

4. Experimental

Melting points are uncorrected and measured in open capillary tubes using a Gallenkamp electric melting point apparatus. IR spectra were recorded on Perkin-Elmer 598 and FT-IR 1650 spectrophotometers, using samples in KBr disks. ^1H NMR spectra were taken on an EM-NMR spectrometer (300 MHz) using $\text{DMSO-}d_6$ or CDCl_3 as solvent and TMS as the internal standard. Mass spectra were obtained on a HP MS-5988 by direct inlet ($E = 70$ eV). Elemental microanalyses were performed at Cairo University, Microanalytical Center.

5-Acetyl-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (1)

A mixture of 4-hydroxy-1-phenylpyrazolo[3,4-d]pyrimidine (0.01 mol) and acetyl chloride (0.01 mol) in dry benzene (10 mL) and triethylamine (one drop) was heated under reflux for 12 h. The reaction mixture was concentrated and cooled. The solid was filtered off and recrystallized to give **1**.¹⁹ IR: ν/cm^{-1} 2980 ($\text{CH}_{\text{aliphatic}}$), 1775 ($\text{C=O}_{\text{acetyl}}$), 1660 ($\text{C=O}_{\text{pyrimidinone}}$), 1610–1600 (C=N , C=C). ^1H NMR ($\text{DMSO-}d_6$) δ 2.56 (s, 3H, COCH_3), 7.31–8.22 (m, 5H, Ar-H), 8.61 (s, 1H, $\text{CH}_{\text{pyrazole}}$), 8.72 (s, 1H, $\text{CH}_{\text{pyrimidinone}}$).

Ethyl 2,4-dioxo-4-(4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)butanoate (2)

A mixture of **1** (0.03 mol), finely divided sodium metal (0.15 mol) and dry diethylxalate (0.68 mol), was refluxed for 4 h. The reaction mixture was kept at room temperature over night, then poured into diluted acetic acid. The precipitate that formed was filtered off, washed with water and recrystallized from EtOH to give **2**, m.p. 170 °C. *Anal.* Calcd for $\text{C}_{17}\text{H}_{14}\text{N}_4\text{O}_5$: C, 57.63; H, 3.95; N, 15.82. Found: C, 57.65; H, 4.00; N, 15.80. IR: ν/cm^{-1} 2980 ($\text{CH}_{\text{aliphatic}}$), 1725 ($\text{C=O}_{\text{ester}}$), 1660, 1650 and 1630 ($\alpha\text{-C=O}$, $\gamma\text{-C=O}$ and $\text{C=O}_{\text{pyrimidinone}}$), 1610–1585 (C=N , C=C). ^1H NMR ($\text{DMSO-}d_6$) δ 1.25 (t, 3H, OCH_2CH_3), 4.32 (q, 2H, OCH_2CH_3), 6.11 (s, 1H, CH=C), 7.31–8.22 (m, 5H, Ar-H), 8.61 (s, 1H, $\text{CH}_{\text{pyrazole}}$), 8.72 (s, 1H, $\text{CH}_{\text{pyrimidinone}}$), 14.42 (s, 1H, exchangeable with D_2O , OH).

Ethyl 5-(4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)-1H-pyrazole-3-carboxylate (3)

A mixture of compound **2** (0.01 mol) and hydrazine hydrate (0.01 mol), in ethanol (10 mL), was refluxed for 4 h. The reaction mixture was left to cool at room temperature and the precipitate that formed was filtered off and recrystallized from DMF to give **3**, m.p. 240 °C. *Anal.* Calcd for $\text{C}_{17}\text{H}_{14}\text{N}_6\text{O}_3$: C, 58.28; H, 4.00; N, 24.00.

Found: C, 58.30; H, 3.98; N, 24.10. IR: ν/cm^{-1} 3167 (NH), 2990–2930 ($\text{CH}_{\text{aliphatic}}$), 1728 ($\text{C=O}_{\text{ester}}$), 1630 ($\text{C=O}_{\text{pyrimidinone}}$), 1610–1585 (C=N , C=C). ^1H NMR ($\text{DMSO-}d_6$) δ 1.25 (t, 3H, OCH_2CH_3), 4.32 (q, 2H, OCH_2CH_3), 6.90–8.25 (m, 7H, Ar-H, C-4- $\text{H}_{\text{pyrazoline}}$, $\text{NH}_{\text{pyrazoline}}$), 8.61 (s, 1H, $\text{CH}_{\text{pyrazole}}$), 8.72 (s, 1H, $\text{CH}_{\text{pyrimidinone}}$).

5-(4-Oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)-1H-pyrazole-3-carbohydrazide (4)

A mixture of compound **3** and/or **2** (0.01 mol) and hydrazine hydrate (0.09 mol), was heated under fusion condition for 1 h, then the reaction mixture was treated with 20 mL of ethanol and the mixture was refluxed for another 4 h. The product formed was collected by filtration and recrystallized from MeOH to give **4**, m.p. 210 °C. *Anal.* Calcd for $\text{C}_{15}\text{H}_{12}\text{N}_8\text{O}_2$: C, 53.57; H, 3.57; N, 33.33. Found: C, 53.51; H, 3.60; N, 33.29. IR: ν/cm^{-1} 3420, 3300 (NH_2), 3163 (NH), 2990 ($\text{CH}_{\text{aliphatic}}$), 1680 ($\text{C=O}_{\text{acid hydrazide}}$), 1628 ($\text{C=O}_{\text{pyrimidinone}}$), 1610–1590 (C=N , C=C). ^1H NMR ($\text{DMSO-}d_6$) δ 3.42 (bs, 2H, NH_2), 7.30–8.25 (m, 7H, Ar-H, C-4- $\text{H}_{\text{pyrazoline}}$, $\text{NH}_{\text{pyrazoline}}$), 8.61 (s, 1H, $\text{CH}_{\text{pyrazole}}$), 8.72 (s, 1H, $\text{CH}_{\text{pyrimidinone}}$), 10.00 (bs, 1H, $\text{NH}_{\text{hydrazide}}$).

5-(4-Oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)-1H-pyrazole-3-carboxylic acid (5)

A solution of compound **3** (0.01 mol) in sodium hydroxide (25 mL, 5%) was heated under reflux on a water bath for 2 h. The clear solution was filtered off from any insoluble materials and neutralized with hydrochloric acid, the solid product that formed was filtered off and recrystallized from EtOH to give **5**, m.p. 260 °C. *Anal.* Calcd for $\text{C}_{15}\text{H}_{10}\text{N}_6\text{O}_3$: C, 55.90; H, 3.10; N, 26.09. Found: C, 55.85; H, 3.15; N, 26.10. IR: ν/cm^{-1} 3168 (NH), 2975–2935 ($\text{CH}_{\text{aliphatic}}$), 2600 (H-bonded OH, the carboxylic OH), 1729 ($\text{C=O}_{\text{carboxylic}}$), 1628 ($\text{C=O}_{\text{pyrimidinone}}$), 1610–1590 (C=N , C=C). ^1H NMR ($\text{DMSO-}d_6$) δ 7.30–8.25 (m, 6H, Ar-H, $\text{H}_{\text{olefinic}}$), 8.61 (s, 1H, $\text{CH}_{\text{pyrazole}}$), 8.72 (s, 1H, $\text{CH}_{\text{pyrimidinone}}$), 9.61 (s, 1H, $\text{NH}_{\text{pyrazoline}}$), 13.52 (bs, 1H, $\text{OH}_{\text{carboxylic}}$).

1-Phenyl-5-(1H-pyrazol-5-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (6)

One g of the acid **5** was heated until it melts and the temperature of the molten phase was kept constant above the melting point of the acid by 283 K for 10 min, then the residue after cooling was treated with 20 mL of ethanol and the solid product that formed was collected and recrystallized from MeOH to give **6**, m.p. 210 °C. *Anal.* Calcd for $\text{C}_{14}\text{H}_{10}\text{N}_6\text{O}$: C, 60.43; H, 3.59; N, 25.89. Found: C, 60.40; H, 3.60; N, 25.91. IR: ν/cm^{-1} 3175 (NH), 2975 ($\text{CH}_{\text{aliphatic}}$), 1630 ($\text{C=O}_{\text{pyrimidinone}}$), 1610–1590 (C=N , C=C). ^1H NMR ($\text{DMSO-}d_6$) δ 7.20–8.20 (m, 7H, Ar-H, 2 $\text{H}_{\text{pyrazole}}$), 8.61 (s, 1H, $\text{CH}_{\text{pyrazole}}$), 8.72 (s, 1H, $\text{CH}_{\text{pyrimidinone}}$), 9.71 (s, 1H, $\text{NH}_{\text{pyrazoline}}$).

5-[3-(1H-Benzimidazol-2-yl)-1H-pyrazol-5-yl]-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (7)

To a solution of compound **3** (0.01 mol) in DMF (30 mL), *o*-phenylenediamine (0.01 mol) was added and the reaction mixture was refluxed for 5 h, afterwards, the mixture was poured into ice-cold water. The precipitate formed was filtered off and recrystallized from EtOH to give

7, m.p. > 300 °C. *Anal.* Calcd for C₂₁H₁₄N₈O: C, 63.96; H, 3.55; N, 28.43. Found: C, 63.94; H, 3.60; N, 28.40. IR: ν/cm^{-1} 3235–3125 (NH), 2975 (CH_{aliphatic}), 1630 (C=O_{pyrimidinone}), 1610–1590 (C=N, C=C). ¹H NMR (DMSO-*d*₆) δ 7.20–8.20 (m, 10H, Ar-H, 1H_{pyrazole}), 8.61 (s, 1H, CH_{pyrazole}), 8.72 (s, 1H, CH_{pyrimidinone}), 9.22 (s, 1H, NH_{imidazole}), 9.73 (s, 1H, NH_{pyrazoline}).

Ethyl 5-(4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)-3-isoxazolecarboxylate (8)

Compound **2** was cured with hydroxylamine utilizing the same procedure described for compound **3**, and worked up as there. The precipitate so formed was filtered off and recrystallized from DMF to give **8**, m.p. 210 °C. *Anal.* Calcd for C₁₇H₁₃N₅O₄: C, 58.12; H, 3.70; N, 19.94. Found: C, 58.10; H, 3.68; N, 19.88. IR: ν/cm^{-1} 2975 (CH_{aliphatic}), 1730 (C=O_{ester}), 1630 (C=O_{pyrimidinone}), 1610–1590 (C=N, C=C), 1044 (C-O-C). ¹H NMR (DMSO-*d*₆) δ 1.28 (t, 3H, OCH₂CH₃), 4.31 (q, 2H, OCH₂CH₃), 7.25–8.21 (m, 6H, Ar-H, 1H_{isoxazole}), 8.61 (s, 1H, CH_{pyrazole}), 8.72 (s, 1H, CH_{pyrimidinone}).

5-(4-Oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)-3-isoxazolecarbohydrazide (9)

A mixture of compound **8** (0.01 mol) and hydrazine hydrate (5 mL, 0.09 mol), was heated under fusion condition (503 K) for 1 h, then the reaction mixture was treated with ethanol (20 mL) and refluxed for 4 h. The reaction mixture was cooled and the precipitate was collected by filtration. The precipitate was dissolved in 10 mL of 3:7 DMF/H₂O at 200 °C. The solution was cooled and the crystalline solid collected by filtration. The solid was washed with cold EtOH and vacuum dried to provide **9** as colorless needles, m.p. 235 °C. *Anal.* Calcd for C₁₅H₁₁N₇O₃: C, 53.41; H, 3.26; N, 29.08. Found: C, 53.88; H, 3.24; N, 29.11. IR: ν/cm^{-1} 3322 (NH₂), 3190 (NH), 2997 (CH_{aliphatic}), 1715 (C=O_{acid hydrazide}), 1625 (C=O_{pyrimidinone}), 1615–1595 (C=N, C=C). ¹H NMR (DMSO-*d*₆) δ 3.41 (bs, 2H, NH₂), 7.25–8.21 (m, 6H, Ar-H, 1H_{isoxazole}), 8.61 (s, 1H, CH_{pyrazole}), 8.72 (s, 1H, CH_{pyrimidinone}), 10.00 (bs, 1H, NH_{hydrazide}).

5-(4-Oxo-4,5-dihydropyrazolo[1,5-d][1,2,4]triazin-2-yl)-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (10)

A mixture of compound **4** (0.01 mol) and triethyl orthoformate (0.015 mol) was heated at the boiling point of the mixture for 2 h, using a short air condenser. The reaction mixture was allowed to cool and treated with 20 mL of diethyl ether. The solid that formed was filtered off and recrystallized from EtOH to give **10**, m.p. 220 °C. *Anal.* Calcd for C₁₆H₁₀N₈O₂: C, 55.49; H, 2.89; N, 32.37. Found: C, 55.50; H, 2.90; N, 32.41. IR: ν/cm^{-1} 3170 (NH), 2970 (CH_{aliphatic}), 1680 (C=O_{triazinone}), 1630 (C=O_{pyrimidinone}), 1610–1590 (C=N, C=C). ¹H NMR (DMSO-*d*₆) δ 7.25–8.25 (m, 7H, Ar-H, CH_{pyrazole}, CH_{triazine}),

8.61 (s, 1H, CH_{pyrazole}), 8.72 (s, 1H, CH_{pyrimidinone}), 9.92 (bs, 1H, NH_{triazine}).

5-(4-Oxo-7-phenyl-4,5-dihydropyrazolo[1,5-d][1,2,4]triazin-2-yl)-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidine-4-one (11)

A mixture of compound **4** (0.01 mol) and benzoyl chloride (0.01 mol) in dry pyridine, was refluxed for 6 h, the product so formed was filtered off, washed with diluted hydrochloric acid and recrystallized from DMF/H₂O to give **11**, m.p. 260 °C. *Anal.* Calcd for C₂₂H₁₄N₈O₂: C, 62.56; H, 3.32; N, 26.54. Found: C, 62.50; H, 3.30; N, 26.50. IR: ν/cm^{-1} 3195 (NH), 2980 (CH_{aliphatic}), 1675 (C=O_{triazinone}), 1625 (C=O_{pyrimidinone}), 1610–1595 (C=N, C=C). ¹H NMR (DMSO-*d*₆) δ 7.20–8.22 (m, 11H, Ar-H, CH_{pyrazole}), 8.61 (s, 1H, CH_{pyrazole}), 8.72 (s, 1H, CH_{pyrimidinone}), 9.90 (bs, 1H, NH_{triazine}).

5-(4-Oxo-7-sulfanyl-4,5-dihydropyrazolo[1,5-d][1,2,4]triazin-2-yl)-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (12)

A mixture of **4** (0.01 mol), carbon disulfide (0.02 mol) and potassium hydroxide (5 mL, 10%) in ethanol (30 mL), was refluxed on a water bath for 4 h, then the mixture was poured into ice-cold ether, acidified with dilute hydrochloric acid and the solid formed was collected and recrystallized from EtOH to give **12**, m.p. 245 °C. *Anal.* Calcd for C₁₆H₁₀N₈O₂S: C, 50.79; H, 2.64; N, 29.63. Found: C, 50.78; H, 2.70; N, 29.60. IR: ν/cm^{-1} 3195–3165 (NH), 2975 (CH_{aliphatic}), 2660 (SH), 1675 (C=O_{triazinone}), 1630 (C=O_{pyrimidinone}), 1610–1590 (C=N, C=C). ¹H NMR (DMSO-*d*₆) δ 1.95 (bs, 1H, SH), 7.25–8.25 (m, 6H, Ar-H, CH_{pyrazole}), 8.61 (s, 1H, CH_{pyrazole}), 8.72 (s, 1H, CH_{pyrimidinone}), 10.40 (bs, 1H, NH_{triazine}).

N'-[(4-Oxo-4H-chromen-3-yl)methylidene]-3-(4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)-1H-pyrazole-5-carbohydrazide (14)

Equimolar amounts of **4** and **13** (0.01 mol) in absolute ethanol (25 mL) was refluxed for 4 h, the product formed was filtered off and recrystallized from MeOH to give **14**, m.p. 170 °C. *Anal.* Calcd for C₂₅H₁₆N₈O₄: C, 60.97; H, 3.25; N, 22.76. Found: C, 60.95; H, 3.22; N, 22.80. IR: ν/cm^{-1} 3190–3175 (NH), 2975 (CH_{aliphatic}), 1680 (HNC=O), 1640 (C=O_{pyrone}), 1630 (C=O_{pyrimidinone}), 1610–1590 (C=N, C=C). ¹H NMR (DMSO-*d*₆) δ 6.55 (s, 1H, CH_{olefinic}), 7.30–8.25 (m, 11H, Ar-H, C-4-H_{pyrazoline}, NH_{pyrazoline}), 8.61 (s, 1H, CH_{pyrazole}), 8.72 (s, 1H, CH_{pyrimidinone}), 9.21 (s, 1H, pyrone), 10.10 (bs, 1H, NH).

3-(4-Oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)-N'-[[3-(4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-d]pyrimidin-5-yl)-1H-pyrazol-5-yl]carbonyl]-1H-pyrazole-5-carbohydrazide (15)

A mixture of compound **4** (0.01 mol) and the ester **3** (0.01 mol) in DMF (10 mL) was heated under reflux for 2

h. Then, the reaction mixture was poured into cold water and the precipitate that formed was filtered off and recrystallized from AcOH/H₂O to give **15**, m.p. 250 °C. *Anal.* Calcd for C₃₀H₂₀N₄O₄: C, 56.25; H, 3.12; N, 30.62. Found: C, 56.20; H, 3.10; N, 30.59. IR: ν /cm⁻¹ 3250–3182 (NH), 2995 (CH_{aliphatic}), 1675 (NHC=O), 1630 (C=O_{pyrimidinone}), 1610–1595 (C=N, C=C). ¹H NMR (DMSO-*d*₆) δ 7.30–8.25 (m, 14H, Ar-H, 2C-4-H_{pyrazoline}, 2NH_{pyrazoline}), 8.61 (s, 2H, CH_{pyrazole}), 8.72 (s, 2H, CH_{pyrimidinone}), 10.00 (bs, 2H, NH_{hydrazide}).

Ethyl 5-(4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-*d*]pyrimidin-5-yl)-1-phenyl-1H-pyrazole-3-carboxylate (16)

Similarly, using the same method as for the preparation of compound **3**, treatment of compound **2** with phenylhydrazine afforded compound **16** which was recrystallized from anisole, m.p. 200 °C. *Anal.* Calcd for C₂₃H₁₈N₆O₃: C, 64.78; H, 4.22; N, 19.71. Found: C, 64.72; H, 4.25; N, 19.70. IR: ν /cm⁻¹ 2975 (CH_{aliphatic}), 1725 (C=O_{ester}), 1630 (C=O_{pyrimidinone}), 1610–1595 (C=N, C=C). ¹H NMR (DMSO-*d*₆) δ 1.25 (t, 3H, OCH₂CH₃), 4.30 (q, 2H, OCH₂CH₃), 6.90 (s, 1H, CH_{pyrazole}), 7.25–8.20 (m, 10H, Ar-H), 8.61 (s, 1H, CH_{pyrazole}), 8.72 (s, 1H, CH_{pyrimidinone}).

5-(4-Oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-*d*]pyrimidin-5-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide (17)

Using the same procedure described for compounds **4** and **9**, treatment of **16** with hydrazine hydrate gave compound **17**, which was recrystallized from EtOH, m.p. 235 °C. *Anal.* Calcd for C₂₁H₁₆N₈O₂: C, 61.16; H, 3.88; N, 27.18. Found: C, 61.20; H, 3.90; N, 27.15. IR: ν /cm⁻¹ 3320–3260, 3178 (NH₂, NH), 2977 (CH_{aliphatic}), 1675 (C=O_{acid hydrazide}), 1630 (C=O_{pyrimidinone}), 1610–1585 (C=N, C=C). ¹H NMR (DMSO-*d*₆) δ 3.28 (bs, 2H, NH₂), 7.32–8.20 (m, 11H, Ar-H, CH_{pyrazoline}), 8.61 (s, 1H, CH_{pyrazole}), 8.72 (s, 1H, CH_{pyrimidinone}), 10.20 (bs, 1H, NH).

2-[[5-(4-Oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-*d*]pyrimidin-5-yl)-1-phenyl-1H-pyrazol-3-yl]carbonyl]hydrazinecarbothioamide (18a) and 2-[[5-(4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-*d*]pyrimidin-5-yl)-1-phenyl-1H-pyrazol-3-yl]carbonyl]-N-phenylhydrazinecarbothioamide (18b)

Method A. To a solution of compound **16** (0.01 mol), in DMF (10 mL), thiosemicarbazide or phenylthiosemicarbazide (0.01 mol) was added and the reaction mixture was refluxed for 6 h. The mixture was then cooled and poured onto crushed ice and the precipitate formed was filtered off and recrystallized from benzene to give **18a**, m.p. 280 °C. *Anal.* Calcd for C₂₈H₂₁N₉O₂S: C, 61.42; H, 3.83; N, 23.03. Found C, 61.30; H, 3.80; N, 23.11. IR: ν /cm⁻¹ 3381, 3276 (NH), 2970 (CH_{aliphatic}), 2660 (SH), 1695 (HNC=O), 1630 (C=O_{pyrimidinone}), 1610–1585 (C=N,

C=C), 1165, 1278 (NHC=S). ¹H NMR (DMSO-*d*₆) δ 7.32–8.20 (m, 17H, Ar-H, CH_{pyrazoline}, CSNH), 8.61 (s, 1H, CH_{pyrazole}), 8.72 (s, 1H, CH_{pyrimidinone}), 9.42 (bs, 1H, CSNH), 10.85 (bs, 1H, CONH).

Method B. To a solution of acid hydrazide **17** (0.01 mol) in hydrochloric acid (10 mL, 10%) and ethanol (20 mL), ammonium thiocyanate (0.012 mol) was added, and the reaction mixture was heated under reflux for 4 h. The mixture was then poured into ice-cold water containing DMF and the obtained precipitate was filtered off and crystallized to produce **18a**.

Method C. To a solution of compound **17** (0.01 mol) in DMF (20 mL), phenylisothiocyanate (0.01 mol) was added. The reaction mixture was refluxed for 2 h, and then poured into ice-cold water. The precipitate formed was filtered off and crystallized to give **18b**.

1-Phenyl-5-[1-phenyl-3-(4-phenyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)-1H-pyrazol-5-yl]-1,5-dihydro-4H-pyrazolo[3,4-*d*]pyrimidin-4-one (19)

To a solution of compound **18b** (0.01 mol) in ethanol (30 mL, 95%), potassium hydroxide (0.015 mol) was added. The reaction mixture was heated under reflux for 4 h. The mixture was filtered and acidified with dilute hydrochloric acid. The precipitate was collected by filtration and recrystallized from DMF to give **19**, m.p. 240 °C. *Anal.* Calcd for C₂₈H₁₉N₉OS: C, 63.52; H, 3.59; N, 23.82. Found C, 63.50; H, 3.60; N, 23.78. IR: ν /cm⁻¹ 3191 (NH), 2970 (CH_{aliphatic}), 1630 (C=O_{pyrimidinone}), 1610–1585 (C=N, C=C), 1396, 1258, 1198 (NHC=S). ¹H NMR (DMSO-*d*₆) δ 7.20–8.20 (m, 16H, Ar-H, CH_{pyrazoline}), 8.61 (s, 1H, CH_{pyrazole}), 8.72 (s, 1H, CH_{pyrimidinone}), 10.60 (bs, 1H, CSNH).

5-[3-(5-phenylamino-1,3,4-thiadiazol-2-yl)-1-phenyl-1H-pyrazol-5-yl]-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-*d*]pyrimidin-4-one (20)

Compound **18b** (0.01 mol) was heated under fusion condition with PPA for 2 h. The mass of the reaction was allowed to cool and poured into cold water containing sodium acetate (20 g). The solid that formed was filtered off and recrystallized from MeOH to give **20**, m.p. 150 °C. *Anal.* Calcd for C₂₈H₁₉N₉OS: C, 63.52; H, 3.59; N, 23.82. Found C, 63.49; H, 3.55; N, 23.80. IR: ν /cm⁻¹ 3250 (NH), 2977 (CH_{aliphatic}), 1630 (C=O_{pyrimidinone}), 1610–1585 (C=N, C=C). ¹H NMR (DMSO-*d*₆) δ 7.30–8.20 (m, 16H, Ar-H, CH_{pyrazoline}), 8.61 (s, 1H, CH_{pyrazole}), 8.72 (s, 1H, CH_{pyrimidinone}), 12.70 (bs, 1H, NH_{thiadiazole}).

5-(4-Oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-*d*]pyrimidin-5-yl)-1-phenyl-1H-pyrazole-3-carboxylic acid (21)

Using the same method described for compound **5**, treatment of **16** with sodium hydroxide (5%) afforded compound **21**, which was recrystallized from MeOH, m.p. 225 °C. *Anal.* Calcd for C₂₁H₁₄N₆O₃: C, 63.32; H, 3.52; N, 21.10. Found C, 63.30; H, 3.55; N, 21.00. IR: ν /cm⁻¹ 2985

(CH_{aliphatic}), 2600 (H-bonded OH, carboxylic OH), 1730 (C=O_{carboxylic group}), 1630 (C=O_{pyrimidinone}), 1610–1595 (C=N, C=C). ¹H NMR (DMSO-*d*₆) δ 7.10–8.25 (m, 11H, Ar-H, CH_{pyrazoline}), 8.61 (s, 1H, CH_{pyrazole}), 8.72 (s, 1H, CH_{pyrimidinone}), 13.50 (bs, 1H, OH_{acid}).

1-Phenyl-5-(1-phenyl-1H-pyrazol-5-yl)-1,5-dihydro-4H-pyrazolo[3,4-*d*]pyrimidin-4-one (22)

Using the same method as for the preparation of compound **6**, compound **21** was subjected to decarboxylation and yielded compound **22**, which was recrystallized from EtOH, m.p. 150 °C. *Anal.* Calcd for C₂₀H₁₄N₆O: C, 67.79; H, 3.95; N, 23.73. Found C, 67.77; H, 3.92; N, 23.70. IR: ν /cm⁻¹ 2990 (CH_{aliphatic}), 1630 (C=O_{pyrimidinone}), 1610–1585 (C=N, C=C). ¹H NMR (DMSO-*d*₆) δ 7.20–8.20 (m, 12H, Ar-H, 2CH_{pyrazoline}), 8.61 (s, 1H, CH_{pyrazole}), 8.72 (s, 1H, CH_{pyrimidinone}).

Ethyl 4-(1,3-benzodioxol-5-yl)-2-oxo-3-[(4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-*d*]pyrimidin-5-yl)carbonyl]-3-butenolate (23)

A mixture of **2** (0.01 mol), piperonal (0.01 mol) and one drop of piperidine was heated on boiling water-bath for 4 h. The reaction mixture was triturated with ethanol and the solid obtained was filtered off, washed with diethyl ether and recrystallized from DMF to give **23**, m.p. 195 °C. *Anal.* Calcd for C₂₅H₁₈N₄O₇: C, 61.73; H, 3.70; N, 11.52. Found C, 61.70; H, 3.69; N, 11.50. IR: ν /cm⁻¹ 2977 (CH_{aliphatic}), 1715 (C=O_{ester}), 1695 (C=O_{α-keto}), 1665 (C=O_{γ-keto}), 1630 (C=O_{pyrimidinone}), 1610–1585 (C=N, C=C), 1033, 1093 (C–O–C). ¹H NMR (DMSO-*d*₆) δ 1.26 (t, 3H, OCH₂CH₃), 4.20 (q, 2H, OCH₂CH₃), 5.55 (s, 2H, OCH₂O), 6.62 (s, 1H, H_{olefinic}), 6.98–8.25 (m, 8H, Ar-H), 8.61 (s, 1H, CH_{pyrazole}), 8.72 (s, 1H, CH_{pyrimidinone}).

Ethyl 4-(1,3-benzodioxol-5-ylmethylene)-5-(4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-*d*]pyrimidin-5-yl)-4H-pyrazole-3-carboxylate (24)

To a solution of compound **23** (0.01 mol) in glacial acetic acid (20 mL), hydrazine hydrate (0.01 mol) was added, the reaction mixture was refluxed for 4 h and poured into ice-cold water, the solid formed was collected and recrystallized from MeOH to give **24**, m.p. 155 °C. *Anal.* Calcd for C₂₅H₁₈N₆O₅: C, 62.24; H, 3.73; N, 17.43. Found C, 62.20; H, 3.75; N, 17.50. IR: ν /cm⁻¹ 2980 (CH_{aliphatic}), 1735 (C=O_{ester}), 1625 (C=O_{pyrimidinone}), 1610–1590 (C=N, C=C), 1033, 1093 (C–O–C). ¹H NMR (DMSO-*d*₆) δ 1.25 (t, 3H, OCH₂CH₃), 4.22 (q, 2H, OCH₂CH₃), 5.95 (s, 2H, OCH₂O), 6.95–8.25 (m, 9H, Ar-H, CH_{olefinic}), 8.61 (s, 1H, CH_{pyrazole}), 8.72 (s, 1H, CH_{pyrimidinone}).

Ethyl 5-(1,3-benzodioxol-5-yl)-4-[(4-oxo-1-phenyl-1,4-dihydro-5H-pyrazolo[3,4-*d*]pyrimidin-5-yl)carbonyl]-4,5-dihydro-1H-pyrazole-3-carboxylate (25)

To a solution of compound **23** (0.01 mol) in absolute ethanol, hydrazine hydrate (0.01 mol) was added and the

mixture was refluxed for 4 h. The reaction mixture was then cooled, and poured into cold water. The formed deposits were filtered off and recrystallized from EtOH/DMF to give **25**, m.p. 140 °C. *Anal.* Calcd for C₂₅H₂₀N₆O₆: C, 60.00; H, 4.00; N, 16.80. Found C, 60.10; H, 3.98; N, 16.77. IR: ν /cm⁻¹ 3250 (NH), 2985 (CH_{aliphatic}), 1730 (C=O_{ester}), 1678 (C=O_{ketonic}), 1630 (C=O_{pyrimidinone}), 1610–1590 (C=N, C=C), 1033, 1093 (C–O–C). ¹H NMR (DMSO-*d*₆) δ 1.26 (t, 3H, OCH₂CH₃), 3.39 (d, 1H, C-5-H_{pyrazoline}), 4.22 (q, 2H, OCH₂CH₃), 5.94 (s, 2H, OCH₂O), 6.55 (d, 1H, C-4-H_{pyrazoline}), 7.10–8.20 (m, 8H, Ar-H), 8.61 (s, 1H, CH_{pyrazole}), 8.72 (s, 1H, CH_{pyrimidinone}), 10.20 (bs, 1H, NH).

5-[3-(1,3-Benzodioxol-5-yl)-7-hydroxy-2,3-dihydro-1H-pyrazolo[3,4-*d*]pyridazin-4-yl]-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-*d*]pyrimidin-4-one (27)

Method A. A mixture of compound **23** (0.01 mol) and hydrazine hydrate (0.02 mol) in absolute ethanol was refluxed for 4 h. The solid formed was filtered off and recrystallized from EtOH to give **27**, m.p. 220 °C. *Anal.* Calcd for C₂₃H₁₆N₈O₄: C, 58.97; H, 3.42; N, 23.93. Found C, 58.95; H, 3.40; N, 23.85. IR: ν /cm⁻¹ 3253–3165 (NH), 2995 (CH_{aliphatic}), ≈2500 (H-bonded OH), 1630 (C=O_{pyrimidinone}), 1610–1590 (C=N, C=C), 1033, 1093 (C–O–C). ¹H NMR (DMSO-*d*₆) δ 3.65 (d, 1H, C-3-H_{pyrazoline}), 5.95 (s, 2H, OCH₂O), 6.90–8.20 (m, 8H, Ar-H), 8.61 (s, 1H, CH_{pyrazole}), 8.72 (s, 1H, CH_{pyrimidinone}), 9.72 (bs, 1H, NH_{pyrazoline}), 9.95 (bs, 1H, NH_{pyrazoline}), 13.50 (bs, 1H, OH).

Method B. A mixture of equimolar amounts of **25** and hydrazine hydrate (0.01 mol) was treated with absolute ethanol. The reaction mixture was then refluxed for 4 h. The solid formed was filtered off and crystallized.

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Povzetek

Pripravljeni sta bila etil 2,4-diokso-4-(4-okso-1-fenil-1,4-dihido-5H-pirazolo[3,4-d]pirimidin-5-il)butanoat in etil 5-(4-okso-1-fenil-1,4-dihido-5H-pirazolo[3,4-d]pirimidin-5-il)-1H-pirazol-3-karboksilat ter njuni kislinski hidrazidi. Te spojine so reagirale s hidrazini, *orto*-fenilendiaminom, trietil ortoformatom, ogljikovim disulfidom in tiosemikarbazidi pri čemer so nastali nekateri novi 5-substituirani pirazolopirimidin-4-oni kot pirazolini, izoksazolini, imidazoli, pirazolotriazini, tiadiazoli in triazoli. Vse nove spojine so pokazale precejšnji učinek na povečanje reaktivnosti celobiaz. Strukture novih spojin so bile dokazane s pomočjo elementnih analiz, IR in ¹H NMR spektrov ter masnih fragmentacij.