Chemistry of Heterocyclic Compounds, Vol. 47, No. 5, August 2011 (Russian original Vol. 47, No. 5, May, 2011)

SUBSTITUTED QUINOLINONES 15*. PREPARATION AND ENZYMATIC ACTIVITY OF SOME PYRAZOLOAZINES LINKED TO THE 4-HYDROXY-1-METHYL-QUINOLIN-2(1*H*)-ONE MOIETY

A. Khodairy[1] and M. Abass[2]**

The heterocyclization reaction of pyrazolinylquinolinone and its 4-chlorobenzylidene, 2-pyridylmethylene, pyridylaminomethylene, and 2-pyridylhydrazone derivatives with some active methylene nitriles and acrylonitriles, is described. These cyclization reactions afforded novel heterocyclic systems, such as pyrazolo[3,4-b]pyridines, pyrano[2,3-c]pyrazoles, and pyrazolo[4,3-c]pyridazines, linked to position 3 of 4-hydroxy-1-methylquinolin-2(1H)-one. The effect of the new products on the activity of α-amylase was examined. Some pyrano[2,3-c]pyrazoles revealed significant increase in α-D-glucose production by the enzyme.

Keywords: pyrano[2,3-*c*]pyrazole, pyrazolinone, pyrazolo[4,3-*c*]pyridazine, pyrazolo[3,4-*b*]pyridine, quinolinone, enzyme regulation.

3-Substituted 4-hydroxyquinolin-2(1H)-one derivatives represent one of the important classes of heterocyclic compounds possessing a wide spectrum of medicinal activity. Biological screening of many prepared and/or natural products of this heterocycle class has shown their antibacterial [2], antitumor [3], anti-HSV [4], antineoplastic [5], anti-inflammatory [6], molluscicidal [7], and herbicidal activity [8]. On the other hand, pyrazolinones have displayed fungicidal [9], antitumor [10], analgesic [11], antipyretic [12], and antineoplastic activity [13]. They have also shown promise for developing congestive heart failure treatments [14]. Pyrazolinones with herbicidal activity, too, have been reported [15]. Fused pyrazoloazines, which are aza isomers of indole, also display significant biological activity. Pyrazolo[3,4-*b*]pyridines are attractive compounds due to their wide range of pharmaceutical applications. Some representatives of this class have displayed important hypotensive, vasodilator, anti-inflammatory, analgesic, and antipyretic activity [16], others are

* For Communication 14, see [1].

** To whom correspondence should be addressed, e-mail: quinolinone@yahoo.com.

[1] Department of Chemistry, Faculty of Science, Sohag University, Sohag 82524, Egypt.

[2] Department of Chemistry, Faculty of Education, Ain Shams University, Cairo 11757, Egypt.

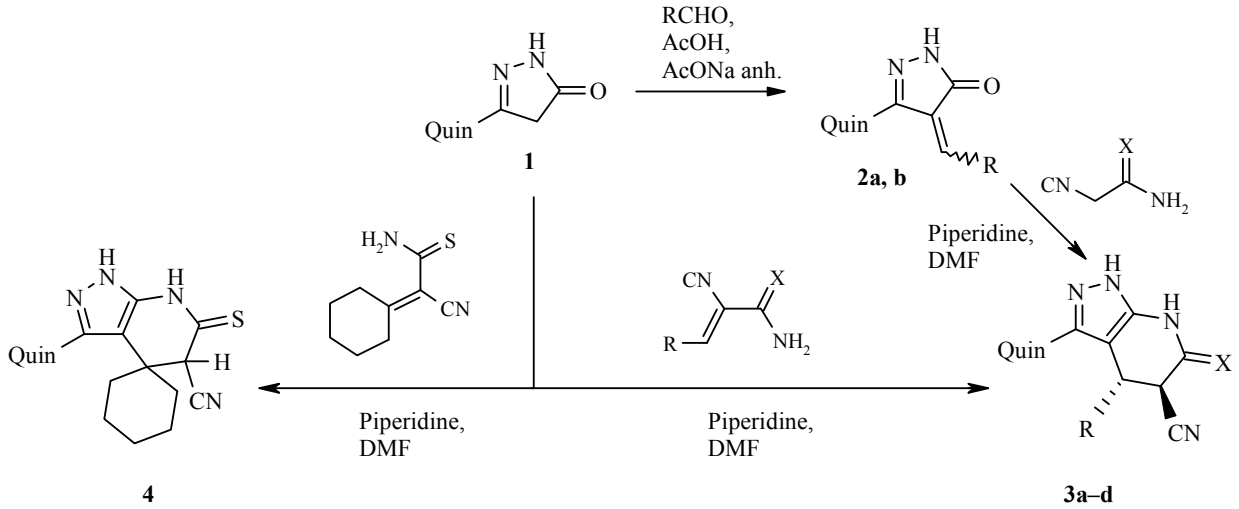
Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 5, pp. 736–749, May, 2011. Received December 9, 2010, in revised form April 3, 2011.

0009-3122/11/4705-0611©2011 Springer Science+Business Media, Inc.

xanthine oxidase [17] or HIV reverse transcriptase inhibitors [18], antitumor agents [19, 20], or anxiolytics [21]. Some pyrano[2,3-*c*]pyrazoles have demonstrated analgesic activity [22], as well as potential inhibitory activity against human kinase Chk1 [23]. Pyrazolo[3,4-*c*]pyridazine derivatives are known to possess interesting biological and pharmacological properties [24–26]. Some of them were identified in high-throughput screening as potent inhibitors of cyclin-dependent kinases CDK1/cyclin-B [27]. In view of these important applications of the three classes of pyrazoloazines it is thought that combination of both quinolinone and some pyrazoloazine moieties in one-molecular frame may lead to a new biologically active series of heterocyclic compounds. In continuation of our program dealing with the synthesis and study of new pyrazoles [28, 29] and substituted quinolinones [30, 31], the present work focused on the synthesis of some fused pyrazoloazine derivatives incorporating the 4-hydroxy-1-methylquinolin-2(1H)-one moiety as the substituent attached to pyrazolinone. An important regulating effect of quinolinones on the activity of α-amylase in efficient conversion of starch to D-glucose has been described previously [32, 33]. These results encouraged us to check the effect of the described new compounds on the activity of this enzyme.

The Knoevenagel condensation reaction of two selected aldehydes, 4-chlorobenzaldehyde and pyridine-2-carbaldehyde, with 4-hydroxy-1-methyl-3-(5-oxo-4,5-dihydro-1H-pyrazol-3-yl)quinolin-2(1H)-one (**1**) in the presence of fused sodium acetate and glacial acetic acid is considered an efficient procedure to obtain 3-[4-arylidene-5-oxo-4,5-dihydro-1H-pyrazol-3-yl]-4-hydroxy-1-methylquinolin-2(1H)-ones **2a** and **2b** [30, 31]. Michael addition of pyrazolinone **1** to 3-aryl-2-cyanoacrylamides and/or thioacrylamides in the presence of piperidine as a base catalyst furnished heterocyclized fused systems, pyrazolo[3,4-*b*]pyridines **3a–d,** in 64–72% yields.

Scheme 1

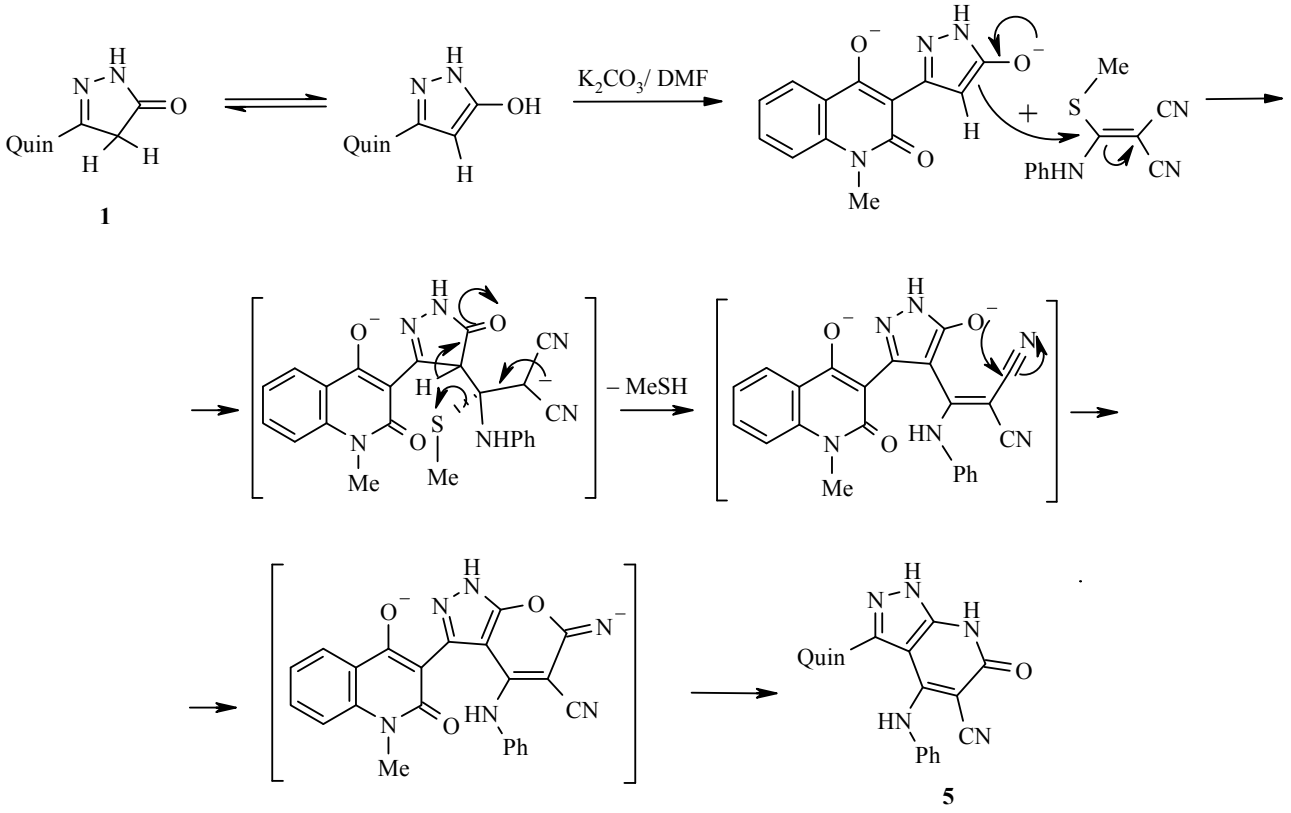


Quin = 4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl;
2a, 3a,b R = 4-chlorophenyl; **2b, 3c,d** R = 2-pyridyl; **3 a,c** X = O, **b,d** X = S

The structure of the products was deduced from their IR, ^{1}H NMR, as well as mass spectra, and elemental analysis. The ^{1}H NMR spectra of compounds **3a–d** indicated the formation of a *trans* isomer. The two protons at positions 4 and 5 of pyrazolo[3,4-*b*]pyridine appear as doublets with coupling constants $J = 11.7 \pm 0.1$ Hz, a characteristic coupling value for transoid protons. As an explanation, getting the *trans* isomer is probably due to increased steric hindrance in its possible *cis* form.

Recently, Rahmati had used the multicomponent condensation reaction technique and obtained related results; even though he had separated both isomers, the major product was the *trans* form [34]. Interestingly, when we subjected compounds **2a**,**b** to Michael addition with cyanoacetamide and cyanothioacetamide in the presence of piperidine, the addition was accompanied by cyclization and we obtained the same products **3a–d** in 76–84% yields. Similarly, the reaction of pyrazolinone **1** with 2-cyano-2-cyclohexylidenethioacetamide in the presence of piperidine led to the spiro[cyclohexane-1,4'-pyrazolo[3,4-*b*]pyridine] derivative **4** (Scheme 1).

Scheme 2

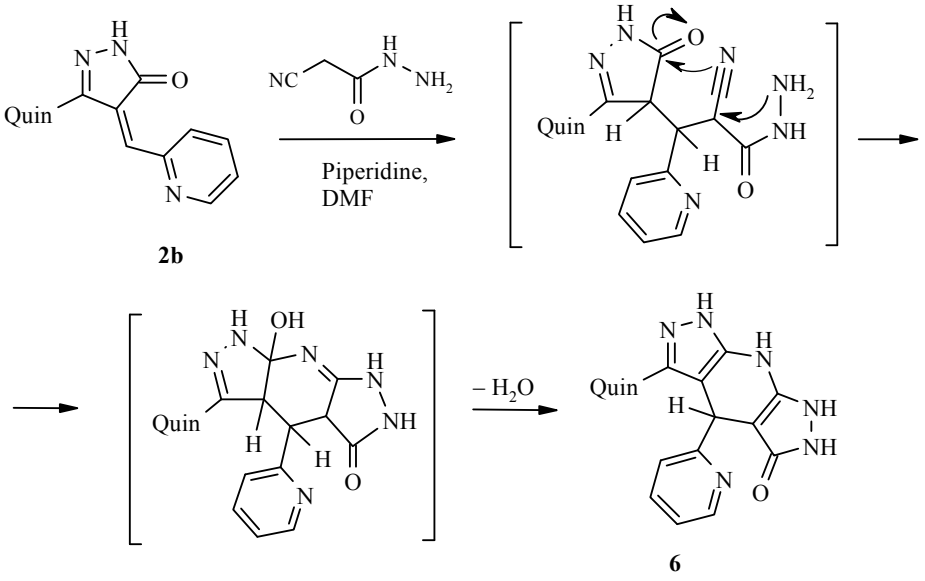


The reaction of pyrazolinone **1** with [anilino(methylthio)methylidene]malononitrile in the presence of dry potassium carbonate in DMF furnished compound **5**. It is likely that a base-catalyzed nucleophilic addition took place to give a tetrahedral adduct intermediate (Scheme 2). This intermediate loses methanethiol to give the enamine intermediate which, in turn, undergoes intramolecular cyclization, followed by iminopyrane–pyridone Dimroth-like rearrangement [35, 36] to give the pyrazolopyridine **5**. The evolution of methanethiol during the course of reaction was detectable, the ^{1}H NMR spectrum of the product revealed four deuterium-exchangeable protons, and elemental analysis showed the absence of sulfur.

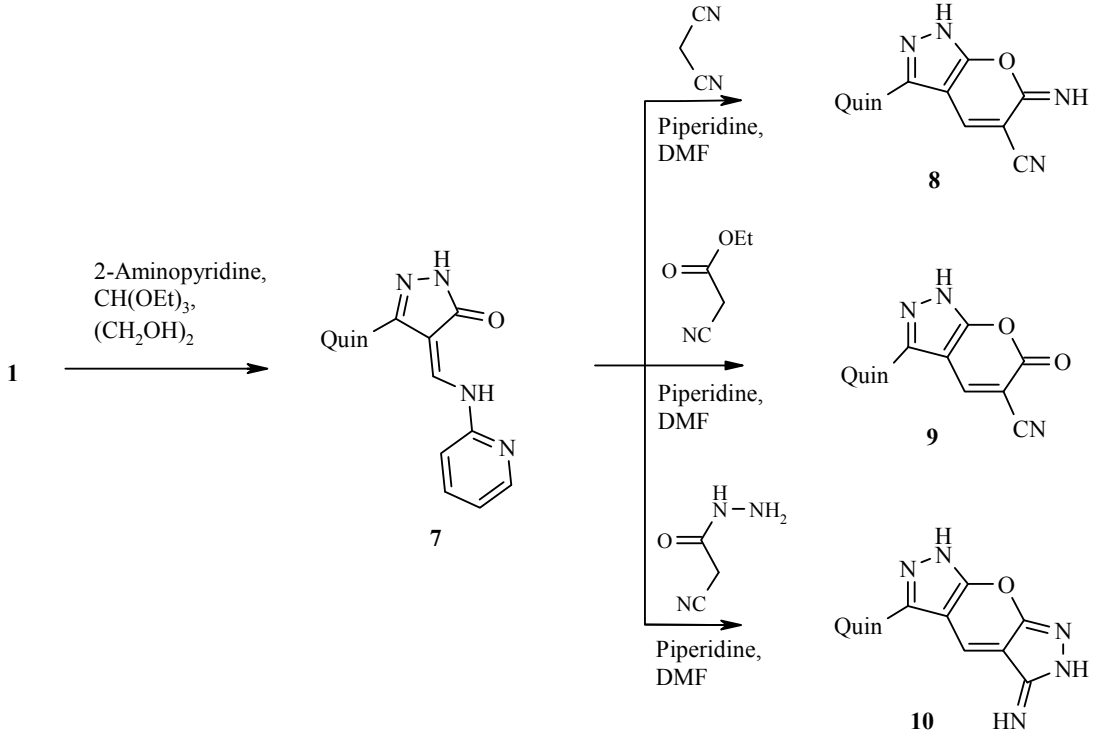
The addition of cyanoacetohydrazide to compound **2b** in the presence of piperidine afforded a triheterocyclic system, dipyrazolopyridine **6**. A possible mechanism for the formation of compound **6** may involve a cascade nucleophilic addition and cyclization with the removal of a water molecule (Scheme 3). The structure of the dipyrazolopyridine **6** was established on the basis of its spectral and elemental analysis data.

4-((Pyridin-2-ylamino)methylidene)pyrazolin-5-one **7** [30], considered as a β-enaminone system, was subjected to Michael addition reaction with some active methylene compounds – malononitrile, ethyl cyanoacetate, and cyanoacetohydrazide – in the presence of piperidine as a base catalyst. Treatment of the compound **7** with malononitrile afforded iminopyranopyrazole **8** (Scheme 4). Similarly, treatment of compound **7** with ethyl cyanoacetate gave pyranopyrazolone **9**. The most important feature of these last two reactions is that addition of the active methylene nitrile at the β-enaminone is accompanied by elimination of 2-aminopyridine. This is followed by intramolecular cyclization *via* nucleophilic addition of the deprotonated pyrazole hydroxy group to either the cyano group, in the case of malononitrile, or the ester group, in the case of ethyl cyanoacetate (Scheme 4). The structure of both compounds **8** and **9** was established from their spectra and elemental analysis data.

Scheme 3



Scheme 4

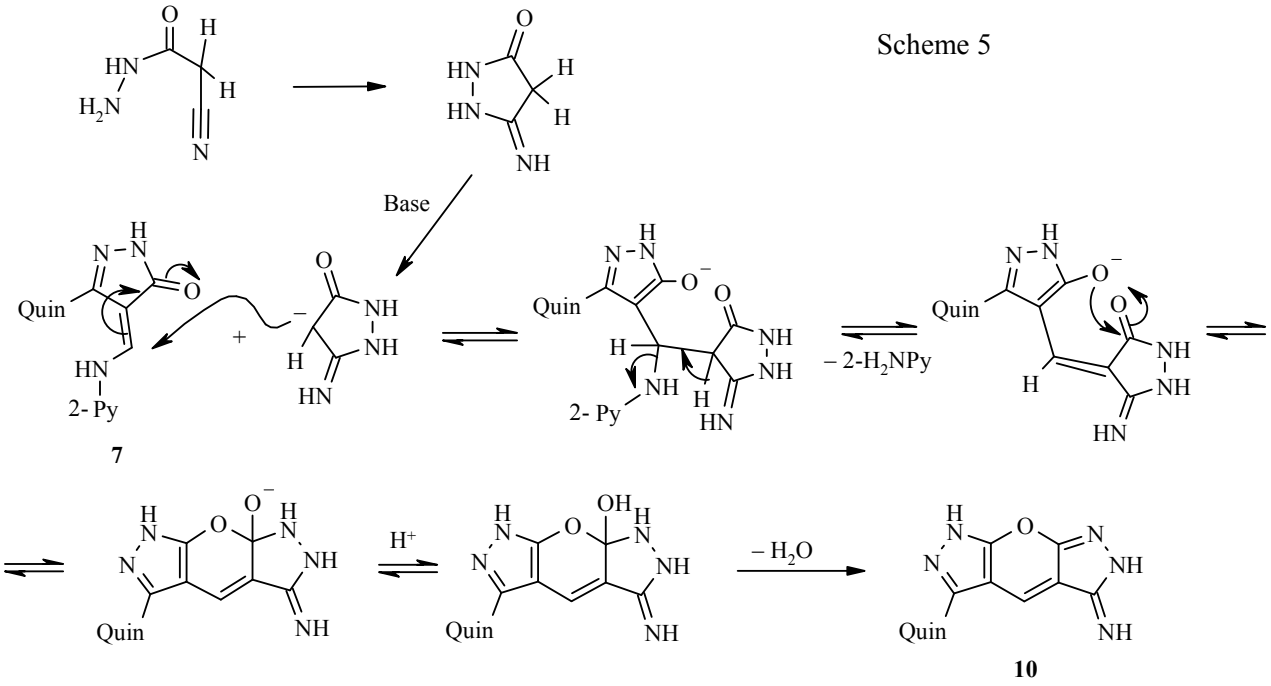


In an analogous way, the reaction of cyanoacetohydrazide with compound **7** is another cascade addition followed by cyclization to give pyrazolo[4',3':5,6]pyrano[2,3-*c*]pyrazole **10**. The IR spectrum of the product showed that, unlike compounds **8** and **9**, its structure does not include a nitrile group, and its proton NMR spectrum has four deuterium-exchangeable protons. We suggest that the addition reaction may start with initial intramolecular cyclization of cyanoacetohydrazide to give iminopyrazolinone. This active methylene-containing

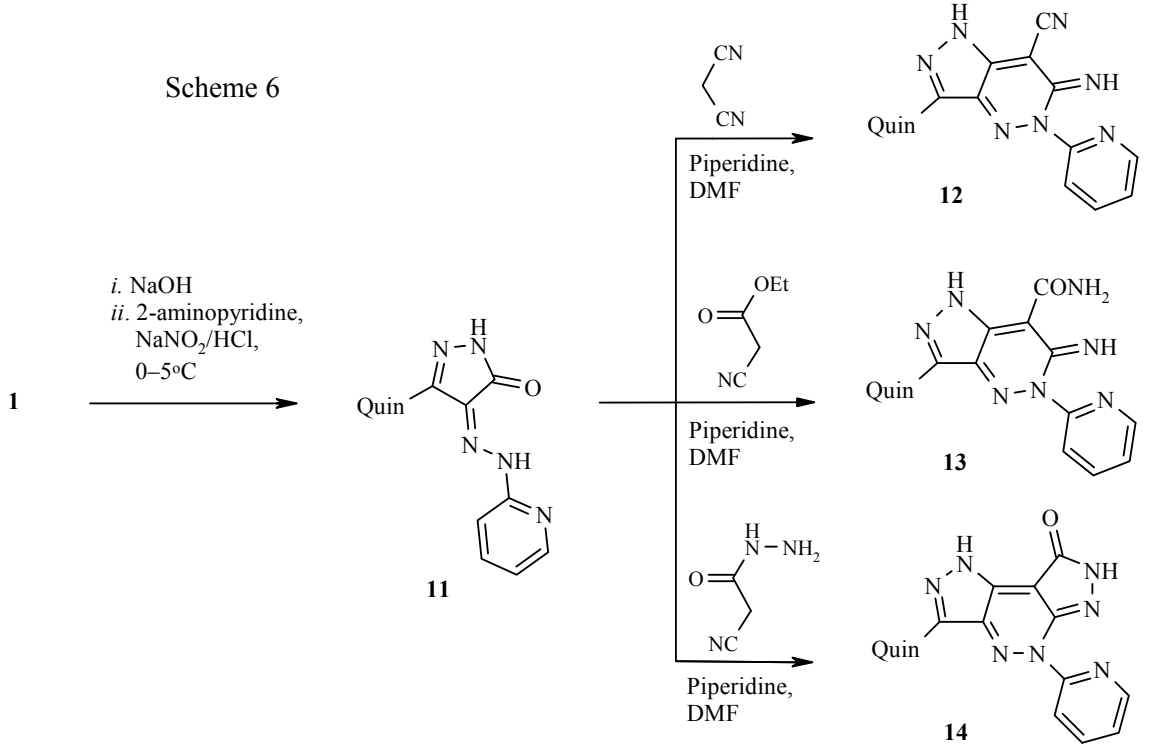
ring adds to the enaminone, and the addition is followed by leaving of a 2-aminopyridine molecule and, finally, cyclization with the loss of a water molecule (Scheme 5).

α-Arylazocarbonyl compounds are considered good precursors for preparation of their corresponding pyridazine derivatives [37]. The behavior of the 4-(pyrid-2-ylhydrazono)pyrazolinone **11** [30], in the presence of piperidine as a base catalyst, towards some nitriles containing an active methylene group was investigated. Thus, treatment of compound **11** with malononitrile furnished pyrazolo[4,3-*c*]pyridazine-7-carbonitrile **12** (Scheme 6).

Scheme 5



Scheme 6

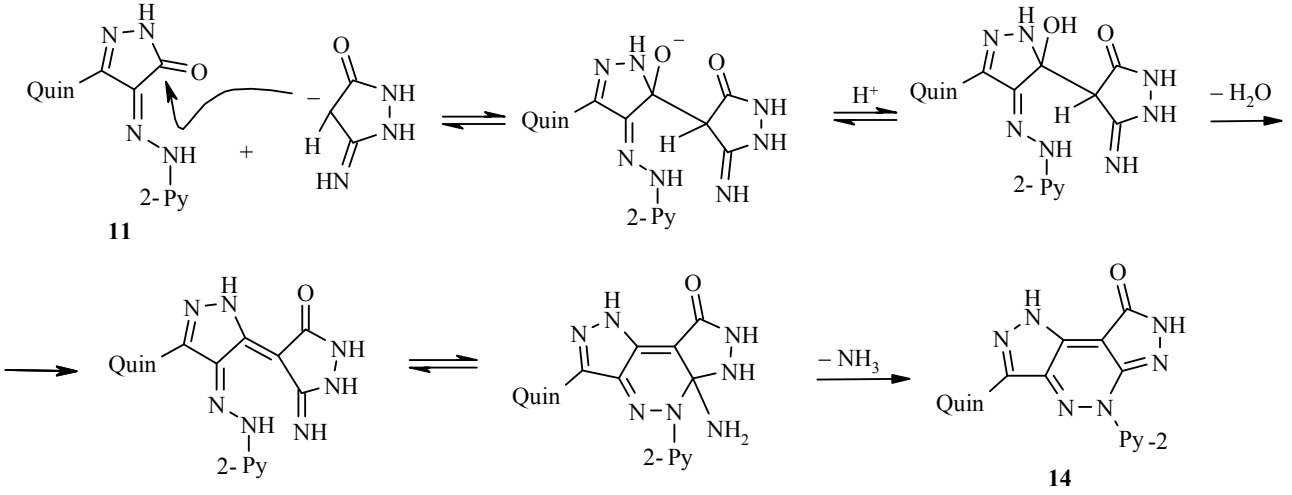


The structure of compound **12** was elucidated through its spectra and elemental analysis. The IR spectrum showed the presence of an absorption due to the C≡N bond at 2214 cm^{-1}, in addition to a sharp strong vibrational band at 1644 cm^{-1} due to C=O of 2-quinolone, while the carbonyl of pyrazolinone was not detected. The ^1H NMR spectrum revealed the existence of three deuterium exchangeable protons at 8.31, 10.30, and 12.60 ppm due to NH and OH groups. Similarly, compound **11** was treated with cyanoacetamide to give the corresponding pyrazolo[4,3-*c*]pyridazine-7-carboxamide **13** in 59% yield. The IR spectrum showed no evidence for the presence of the nitrile function, indicating its involvement in the cyclization reaction, while characteristic vibrations due to the amino group were observed. The ^1H NMR spectrum contained a broad singlet at 6.05 ppm due to the NH_2 protons, which disappeared on addition of D_2O.

A triheterocyclic system, dipyrazolo[3,4-*c*:3',4'-*e*]pyridazinone **14,** was obtained by treating the hydrazone **11** with cyanoacetohydrazide under the above conditions. We suppose that iminopyrazolinone was initially formed by intramolecular cyclization and this active methylene compound attacked the carbonyl carbon of pyrazolinone with the removal of a water molecule. A subsequent cyclization took place accompanied by loss of an ammonia molecule to give the dipyrazolopyridazinone **14** (Scheme 7).

The effect of the new compounds, **3a**–**d**, **4**–**6**, **8**–**10**, and **12**–**14**, on the activity of α-amylase in the hydrolysis of starch was indicated by monitoring production of α-D-glucose. Screening of the new compounds showed that pyranopyrazoles **8**–**10** are the most potent enzyme activator candidates, achieving 5–6-fold increase in the enzyme activity, while pyrazolopyridazines **11**–**14** have moderate effects (Table). It is noted that all the tested compounds activate α-amylase except for compounds **3c** and **3d**, which turned out to be inhibitors and reduced the activity by about 30%. Both compounds possess a pyridyl group, which increases their basicity. However, the importance of this factor in the regulation of this enzyme is not presently known.

Scheme 7



To conclude, this research work describes efficient and facile heterocyclization reactions with pyrazolin-5-one and its 4-arylidene, 4-arylaminomethylene, and 4-arylazo derivatives. Active methylene nitriles and acrylonitriles can be used in such cyclization reaction to obtain the heterocyclic systems, such as pyrazolo-[3,4-*b*]pyridines, pyrano[2,3-*c*]pyrazoles, and pyrazolo[4,3-*c*]pyridazines, linked to position 3 of 4-hydroxy-1-methylquinolin-2(1H)-one. Some quinolinylpyrano[2,3-*c*]pyrazoles revealed a significant effect on the activity of α-amylase.

TABLE 1. Effect of the New Compounds on the Activity of α-Amylase, Indicated by the Amount of α-D-Glucose Produced

Compound	Glucose produced, μg/ml	Enhancement ratio (test/control)
3a	0.45	1.25
3b	0.51	1.42
3c	0.26	0.72
3d	0.25	0.69
4	0.78	2.17
5	1.20	3.33
6	0.28	0.78
8	2.19	6.08
9	1.88	5.22
10	1.76	4.89
12	0.39	1.08
13	0.43	1.19
14	0.54	1.50
Control (DMF)	0.36	–

EXPERIMENTAL

Melting points were determined in open capillary tubes on a digital Stuart SMP3 apparatus. IR spectra were recorded on Perkin-Elmer FT-IR 1650 or Nicolet 710 FT-IR spectrometers using samples in KBr disks. ^{1}H NMR spectra were recorded on a Varian Gemini-200 NMR spectrometer (200 MHz), using TMS as internal reference. Electron-impact mass spectra were determined on a Carlo-Erba QMD-1000 instrument by direct inlet, operating at 70 eV. Elemental analyses were performed on a Perkin Elmer CHN-2400 Analyzer. All reactions were monitored by TLC on 0.2 mm silica gel F-254 (Merck) plates, using UV light (254 and 366 nm) for detection. Compounds **1**, **2a**,**b**, **7**, and **11** were synthesized according to the method described previously [30].

Quinolinylpyrazolopyridines 3a–d (General Method). A. A mixture of the pyrazolinone **1** (1.29 g, 5 mmol), the appropriate 3-aryl-2-cyanoacrylamide (5 mmol), and piperidine (0.5 ml) in DMF (25 ml) was heated under reflux for 4 h. The reaction mixture was left to cool, and the solid precipitate was collected by filtration, washed with cold methanol (20 ml), dried, and crystallized to give compounds **3a–d**, respectively.

B. To a solution of the arylmethylenepyrazolinones **2a** (1.9 g, 5 mmol) or **2b** (1.73 g, 5 mmol) in DMF (30 ml), containing piperidine (0.2 ml), the appropriate active methylene compound (5 mmol) was added. The reaction mixture was heated under reflux for 4 h. After cooling to room temperature, the obtained precipitate was filtered and crystallized from an appropriate solvent to give the corresponding product **3a–d**.

***trans*-4-(4-Chlorophenyl)-3-(4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-6-oxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (3a).** Brown crystals from dioxane, yield 1.60 g (72%) (procedure A), 1.78 g (80%) (procedure B); mp 209–211°C. IR spectrum, ν, cm^{-1}: 1652 (C=O), 1680 (C=O), 2204 (C≡N), 3100–3209 (N–H), 3407 (O–H). ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 3.63 (3H, s, CH_3); 3.95 (1H, d, *J* = 11.7, H-5); 4.44 (1H, d, *J* = 11.6, H-4); 6.98–8.07 (8H, m, H Ar); 9.60 (1H, s, exch. with D_2O, NH); 10.34 (1H, s, exch. with D_2O, NH); 12.62 (1H, s, exch. with D_2O, OH). Mass spectrum, *m/z* (I_{rel}, %): 445 $[M]^+$ (50). Found, %: C 61.60; H 3.40; N 15.80. $C_{23}H_{16}ClN_5O_3$. Calculated, %: C 61.96; H 3.60; N 15.71.

***trans*-4-(4-Chlorophenyl)-3-(4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-6-thioxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (3b).** Yellow crystals from ethanol, yield 1.64 g (71%) (procedure A), 1.93 g (84 %) (procedure B); mp 222–224°C. IR spectrum, ν, cm^{-1}: 1190 (C=S), 1650 (C=O), 2215 (C≡N), 3189–3317 (N–H), 3411 (O–H). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 3.55 (3H, s, CH_3); 3.87 (1H, d, *J* = 11.8, H-5); 4.35 (1H, d, *J* = 11.8, H-4); 6.92–8.04 (9H, m, H Ar + NH); 8.31 (1H, br. s, exch.

with D_2O, NH); 12.06 (1H, s, exch. with D_2O, OH). Mass spectrum, *m/z* (I_{rel}, %): 461 $[M]^+$ (28). Found, %: C 59.60; H 3.20; N 15.00. $C_{23}H_{16}ClN_5O_2S$. Calculated, %: C 59.80; H 3.49; N 15.16.

***trans*-3-(4-Hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-6-oxo-4-pyrid-2-yl-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (3c).** Pale-yellow crystals from methanol, yield 1.34 g (65%) (procedure A), 1.60 g (78%) (procedure B); mp 223–225°C. IR spectrum, ν, cm^{-1}: 1639 (C=O), 1682 (C=O), 2205 (C≡N), 3175–3209 (N–H), 3402 (O–H). ^1H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 3.54 (3H, s, CH_3); 3.96 (1H, d, *J* = 11.6, H-5); 4.28 (1H, d, *J* = 11.7, H-4); 7.02–8.10 (9H, m, H Ar + NH); 10.15 (1H, s, exch. with D_2O, NH); 11.10 (1H, s, exch. with D_2O, OH). Mass spectrum, *m/z* (I_{rel}, %): 412 $[M]^+$ (16). Found, %: C 64.10; H 3.60; N 20.20. $C_{22}H_{16}N_6O_3$. Calculated, %: C 64.07; H 3.91; N 20.38.

***trans*-3-(4-Hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-4-pyrid-2-yl-6-thioxo-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (3d).** Pale-yellow crystals from ethanol, yield 1.37 g (64%) (procedure A), 1.63 g (76%) (procedure B); mp >300°C. IR spectrum, ν, cm^{-1}: 1178 (C=S), 1639 (C=O), 2209 (C≡N), 3177–3209 (N–H), 3405 (O–H). ^1H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 3.63 (3H, s, CH_3); 3.96 (1H, d, *J* = 11.7, H-5); 4.40 (1H, d, *J* = 11.7, H-4); 7.00–8.08 (9H, m, H Ar + NH); 10.31 (1H, br. s, exch. with D_2O, NH); 11.38 (1H, s, exch. with D_2O, OH). Mass spectrum, *m/z* (I_{rel}, %): 428 $[M]^+$ (15). Found, %: C 61.40; H 3.90; N 19.50. $C_{22}H_{16}N_6O_2S$. Calculated, %: C 61.67; H 3.76; N 19.61.

3'-(4-Hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-6'-thioxo-1',5',6',7'-tetrahydrospiro[cyclohexane-1,4'-pyrazolo[3,4-*b*]pyridine]-5'-carbonitrile (4). A mixture of the pyrazolinone **1** (1.29 g, 5 mmol), 2-cyano-2-cyclohexylidenethioacetamide (0.9 g, 5 mmol), and piperidine (0.2 ml) in DMF (20 ml) was heated under reflux for 4 h. The reaction mixture was left overnight, and the solid precipitate was collected by filtration, washed with cold methanol (20 ml), dried, and recrystallized from DMF to give white crystals. Yield 1.70 g (81%); mp 202–204°C. IR spectrum, ν, cm^{-1}: 1176 (C=S), 1650 (C=O), 2206 (C≡N), 3150–3200 (N–H), 3422 (O–H). ^1H NMR spectrum ($CDCl_3$), δ, ppm: 1.32–1.78 (6H, m, 3 CH_2); 2.25–2.49 (4H, m, 2 CH_2); 3.50 (3H, s, CH_3); 3.94 (1H, s, H-5'); 6.50–7.90 (5H, m, H Ar + NH); 10.20 (1H, br. s, exch. with D_2O, NH); 11.53 (1H, s, exch. with D_2O, OH). Mass spectrum, *m/z* (I_{rel}, %): 419 $[M]^+$ (53). Found, %: C 62.60; H 4.90; N 16.40**.** $C_{22}H_{21}N_5O_2S$. Calculated, %: C 62.99; H 5.05; N 16.69.

4-Anilino-3-(4-hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-6-oxo-6,7-dihydro-1H-pyrazolo-[3,4-*b*]pyridine-5-carbonitrile (5). A mixture of the pyrazolinone **1** (1.29 g, 5 mmol), [anilino(methylthio)methylidene]malononitrile (1.08 g, 5 mmol), and anhydrous potassium carbonate (2.76 g) in DMF (25 ml) was heated under reflux for 6 h. The reaction mixture was filtered while hot. The filtrate was treated with diluted hydrochloric acid (5 ml, 1M). The precipitated material obtained was collected by filtration, washed with cold methanol (10 ml), dried, and crystallized from DMF to give dark-brown crystals. Yield 1.36 g (64%); mp 294–295°C. IR spectrum, ν, cm^{-1}: 1650 (C=O), 1682 (C=O), 2202 (C≡N), 3175–3209 (N–H), 3420 (O–H). ^1H NMR spectrum (DMSO-d_6), δ, ppm: 3.68 (3H, s, CH_3); 6.95–7.80 (8H, m, H Ar); 7.90 (1H, s, exch. with D_2O, NH); 8.08 (1H, d, *J* = 8.0, H Ar); 9.10, 9.35 (2H, br. s, exch. with D_2O, 2NH); 12.03 (1H, s, exch. with D_2O, OH). Mass spectrum, *m/z* (I_{rel}, %): 424 $[M]^+$ (17). Found, %: C 65.00; H 3.50; N 19.60. $C_{23}H_{16}N_6O_3$ Calculated, %: C 65.09; H 3.80; N 19.80**.**

5-(4-Hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-4-(pyrid-2-yl)-1,4,7,8-tetrahydrodipyrazolo-[3,4-*b*:4',3'-*e*]pyridin-3(2H)-one (6). A mixture of compound **2b** (1.73 g, 5 mmol), cyanoacetohydrazide (0.5 g, 5 mmol), and piperidine (0.5 ml) in DMF (25 ml) was heated under reflux for 6 h. The reaction mixture was left overnight, and the solid precipitate was collected by filtration, washed with cold methanol (20 ml), dried, and crystallized from dioxane to give light-brown crystals. Yield 1.39 g (65%); mp 294–296°C. IR spectrum, ν, cm^{-1}: 1639 (C=O), 1683 (C=O), 3151–3225 (N–H), 3300 (O–H). ^1H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 3.62 (3H, s, CH_3); 5.04 (1H, s, H-5); 7.06–7.90 (9H, m, H Ar + 2 NH); 8.09 (1H, d, *J* = 7.8, H Ar); 10.30–10.70 (2H, br. s, exch. with D_2O, 2NH); 12.45 (1H, s, exch. with D_2O, OH). Mass spectrum, *m/z* (I_{rel}, %): 427 $[M]^+$ (11). Found, %: C 61.60; H 3.80; N 22.70. $C_{22}H_{17}N_7O_3$. Calculated, %: C 61.82; H 4.01; N 22.94.

Reaction of Active Methylene Compounds with Enaminone 7 (General Method). To a solution of the enaminone **7** (1.80 g, 5 mmol) in DMF (20 ml) containing piperidine (0.2 ml), the appropriate active methylene compound (5 mmol) was added. The reaction mixture was heated under reflux for 4 h. After cooling

at room temperature and dilution with cold water (20 ml), the precipitate was filtered and crystallized from an apropriate solvent to give the pyranopyrazoles **8**, **9**, and **10**, respectively.

3-(4-Hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-6-imino-1,6-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (8). Yellow crystals from methanol, yield 0.92 g (55%); mp 213–215°C. IR spectrum, ν, cm^{-1}: 1608 (C=N), 1639 (C=O), 2205 (C≡N), 3175–3209 (N–H), 3402 (O–H). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 3.48 (3H, s, CH_3); 6.70 (1H, br. s, exch. with D_2O, NH); 7.28–8.05 (5H, m, H Ar); 9.50 (1H, br. s, exch. with D_2O, NH); 11.36 (1H, s, exch. with D_2O, OH). Mass spectrum, *m/z* (I_{rel}, %): 333 $[M]^+$ (35). Found, %: C 61.10; H 3.40; N 20.80. $C_{17}H_{11}N_5O_3$ Calculated, %: C 61.26; H 3.33; N 21.01.

3-(4-Hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-6-oxo-1,6-dihydropyrano-[2,3-*c*]pyrazole-5-carbonitrile (9). Amber-yellow crystals from ethanol, yield 1.33 g (80%); mp 272–274°C. IR spectrum, ν, cm^{-1}: 1638 (C=O), 1712 (C=O), 2200 (C≡N), 3155 (N–H), 3408 (O–H). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 3.68 (3H, s, CH_3); 7.32–8.12 (5H, m, H Ar); 9.90 (1H, s, exch. with D_2O, NH); 11.54 (1H, s, exch. with D_2O, OH). Mass spectrum, *m/z* (I_{rel}, %): 334 $[M]^+$ (13). Found, %: C 61.30; H 3.33; N 16.50. $C_{17}H_{10}N_4O_4$. Calculated, %: C 61.08; H 3.02; N 16.76.

4-Hydroxy-3-(5-imino-5,6-dihydro-1H-pyrazolo[4',3':5,6]pyrano[2,3-*c*]pyrazol-3-yl)-1-methylquinolin-2(1H)-one (10) was crystallized from acetic acid and appears as brown crystals, yield 1.12 g (64%); mp 250–252°C. IR spectrum, ν, cm^{-1}: 1602 (C=N), 1639 (C=O), 3100–3300 (N–H), 3426 (O–H). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 3.48 (3H, s, CH_3); 6.80–7.96 (5H, m, H Ar); 8.40 (1H, s, exch. with D_2O, NH); 9.52–9.78 (2H, br. s, exch. with D_2O, 2 NH); 11.30 (1H, s, exch. with D_2O, OH). Mass spectrum, *m/z* (I_{rel}, %): 348 $[M]^+$ (26). Found, %: C 58.50; H 3.30; N 24.00. $C_{17}H_{12}N_6O_3$. Calculated, %: C 58.62; H 3.47; N 24.13.

Reaction of Active Methylene Compounds with Hydrazonopyrazolinone 11 (General Method). To a solution of the hydrazone **11** (1.81 g, 5 mmol) in DMF (20 ml) containing piperidine (0.2 ml), the appropriate active methylene compound (5 mmol) was added. The reaction mixture was heated under reflux for 4 h. After cooling at room temperature, the precipitate that formed was filtered and crystallized from an appropriate solvent to give the pyrazolopyridazines **12**, **13**, and **14**, respectively.

3-(4-Hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-6-imino-5-pyrid-2-yl-5,6-dihydro-1H-pyrazolo[4,3-*c*]pyridazine-7-carbonitrile (12). White crystals from dioxane, yield 1.27 g (62%); mp 253–254°C. IR spectrum, ν, cm^{-1}: 1644 (C=O), 2214 (C≡N), 3156–3204 (N–H), 3400 (O–H). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 3.63 (3H, s, CH_3); 7.24–8.15 (8H, m, H Ar); 8.31 (1H, s, exch. with D_2O, NH); 10.30 (1H, br. s, exch. with D_2O, NH); 12.60 (1H, s, exch. with D_2O, OH). Mass spectrum, *m/z* (I_{rel}, %): 410 $[M]^+$ (33). Found, %: C 61.30; H 3.70; N 27.50. $C_{21}H_{14}N_8O_2$. Calculated, %: C 61.46; H 3.44; N 27.30.

3-(4-Hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-6-imino-5-pyrid-2-yl-5,6-dihydro-1H-pyrazolo[4,3-*c*]pyridazine-7-carboxamide (13). Pale-yellow crystals from DMF, yield 1.26 g (59%); mp 272–274°C. IR spectrum, ν, cm^{-1}: 1640 (C=O), 1677 (C=O), 3156–3221 (N–H), 3340–3430 (O–H, NH_2). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 3.62 (3H, s, CH_3); 6.05 (2H, br. s, exch. with D_2O, NH_2); 7.22–8.18 (8H, m, H Ar); 8.22 (1H, s, exch. with D_2O, NH); 9.32 (1H, s, exch. with D_2O, NH); 12.60 (1H, s, exch. with D_2O, OH). Mass spectrum), *m/z* (I_{rel}, %): 428 $[M]^+$ (64). Found, %: C 58.60; H 3.60; N 26.00. $C_{21}H_{16}N_8O_3$. Calculated, %: C 58.88; H 3.76; N 26.16.

3-(4-Hydroxy-1-methyl-2-oxo-1,2-dihydroquinolin-3-yl)-5-pyrid-2-yl-5,7-di-hydrodipyrazolo[3,4-*c*:3',4'-*e*]pyridazin-8(1H)-one (14). Greenish-brown crystals from dioxane, yield 1.40 g (66%); mp >300°C. IR spectrum, ν, cm^{-1}: 1639 (C=O), 1680 (C=O), 3170–3209 (N–H), 3402 (O–H). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 3.56 (3H, s, CH_3); 7.24–8.08 (7H, m, H Ar); 8.18 (1H, d, *J* = 7.8, Ar); 9.24 (1H, br. s, exch. with D_2O, NH); 11.30 (1H, br. s, exch. with D_2O, NH); 12.60 (1H, s, exch. with D_2O, OH). Mass spectrum, *m/z* (I_{rel}, %): 426 $[M]^+$ (51). Found, %: C 58.90; H 3.20; N 26.00. $C_{21}H_{14}N_8O_3$. Calculated, %: C 59.15; H 3.31; N 26.28.

Examination of the Effect on α-Amylase. The procedure for determination of the effect of the tested compounds on α-amylase activity was based on measuring the amount of α-D-glucose (μg/ml) that liberated from enzymatic hydrolysis of starch using the standard enzymatic glucose oxidase method [38, 39]. The tests were carried out on α-amylase enzyme produced by *Thermomyces lanuginosus*, a thermophilic fungus which is

grown on starch-containing substances. The tested samples were added as a DMF solution (0.1 ml, 100 μg/ml) to an assay mixture consisting of enzyme solution (0.5 ml) and citrate phosphate buffer (4.5 ml, pH 5.0), containing 1% starch. Then it was incubated at 40°C for 30 min, and the released α-D-glucose was determined on a Spekol-k colorimeter at λ = 505 nm. Tests were carried out in triplicates, and the mean reading was recorded. A blank test was prepared using distilled water instead of the enzyme, and it was used for zero-setting of the colorimeter. A control test was prepared using DMF without the addition of the candidate compounds.

The authors thank Prof. Dr. Usama F. Ali, Department of Biology, Faculty of Education, Ain Shams University, Cairo, Egypt, for evaluation of the enzymatic activity.

REFERENCES

1. M. Abass and A. Khodairy, *Phosphorus*, *Sulfur*, *Silicon*, *Relat. Elem.*, **186**, 287 (2011).
2. M. Azad, M. A. Munawar, and M. Athar, *J. Appl. Sci.*, **7**, 1620 (2007).
3. O. Jansen, V. Akhmedjanova, L. Angenot, G. Balansard, A. Chariot, E. Ollivier, M. Tits, and M. Frédérich, *J. Ethnopharmacol.*, **105**, 241 (2006).
4. A. Afonso, J. Weinstein, and M. J. Gentles, US Pat. 5382572 (1995). http://www.freepatentsonline.com/5382572.pdf
5. B. Podeszwa, H. Niedbala, J. Polanski, R. Musiol, D. Tabak, J. Finster, K. Serafin, M. Milczarek, J. Wietrzyk, S. Boryczka, W. Mol, J. Jampilek, J. Dohnal, D. S. Kalinowski, and D. R. Richardson, *Bioorg. Med. Chem. Lett.*, **17**, 6138 (2007).
6. I. V. Ukrainets, S. G. Taran, L. V. Sidorenko, O. V. Gorokhova, A. A. Ogirenko, A. V. Turov, and N. I. Filimonova, *Chem. Heterocycl. Comp.*, **32**, 960 (1996). [*Khim.Geterotsikl. Siedin.*, 1113 (1996)].
7. M. Abass and B. B. Mostafa, *Bioorg. Med. Chem.*, **13**, 6133 (2005).
8. R. Musiol, D. Tabak, H. Niedbala, B. Podeszwa, J. Jampilek, K. Kralova, J. Dohnal, J. Finster, A. Mencel, and J. Polanski, *Bioorg. Med. Chem.*, **16**, 4490 (2008).
9. K. Findeisen and P.-E. Frohberger, US Pat. 3686410 (1972). http://www.freepatentsonline.com/3686410.pdf
10. M. J. Drysdale, B. W. Dymock, X. Barril-Alonso, P. Workman, L. H. Pearl, C. Prodromou, and E. McDonald, US Pat. 7247734 (2007). http://www.freepatentsonline.com/7247734.pdf
11. A. Gürsoy, Ş. Demirayak, G. Çapan, K. Erol, and K. Vural, *Eur. J. Med. Chem.* **35**, 359 (2000).
12. B. Hinz, O. Cheremina, J. Bachmakov, B. Renner, O. Zolk, M. F. Fromm, and K. Brune, *FASEB J.*, **21**, 2343 (2007).
13. R. Pamukcu and G. A. Piazza, US Pat. 6034099 (2000). http://www.freepatentsonline.com/6034099.pdf
14. D. E. Kuhla, H. F. Campbell, W. L. Studt, and W. C. Faith, US Pat. 4954494 (1990). http://www.freepatentsonline.com/4954494.pdf
15. S. S. Szucs and K.-J. Gu, US Pat. 6465395 (2002). http://www.freepatentsonline.com/6465395.pdf
16. S. Kuribayashi, K. Goto, S. Naito, T. Kamataki, and H. Yamazaki, *Chem. Res. Toxicol.*, **22**, 323 (2009).
17. B. M. Lynch, M. A. Khan, H. C. Teo, and F. Pedrotti, *Can. J. Chem.*, **66**, 420 (1988).
18. S. Saggar, J. T. Sisko, T. J. Tucker, R. M. Tynebor, D. Su, and N. J. Anthony, US Pat. 2007/021442 (2007). http://www.freepatentsonline.com/20070021442.pdf
19. Y. D. Wang, E. Honores, B. Wu, S. Johnson, D. Powell, M. Miranda, J. P. McGinnis, C. Discafani, S. K. Rabindran, W. Cheng, and G. Krishnamurthy, *Bioorg. Med. Chem.*, **17**, 2091 (2009).
20. N. R. Mohamed, N. Y. Khaireldin, A. F. Fahmy, and A. A. El-Sayed, *Der Pharma Chemica*, **2**, 400 (2010). http://derpharmachemica.com/vol2-iss1/DPC-2010-2-1-400-417.pdf
21. B. A. Meiners and A. I. Salama, *Eur. J. Pharmacol.*, **78**, 315 (1982).
22. S. C. Kuo, L. J. Huang, and H. Nakamura, *J. Med. Chem.*, **27**, 539 (1984).

23. N. Foloppe, L. M. Fisher, R. Howes, A. Potter, A. G. S. Robertson, and A. E. Surgenor, *Bioorg. Med. Chem.*, **14**, 4792 (2006).
24. J. Witherington, V. Bordas, A. Gaiba, N. S. Garton, A. Naylor, A. D. Rawlings, B. P. Slingsby, D. G. Smith, A. K. Takle, and R. W. Ward, *Bioorg. Med. Chem. Lett.*, **13**, 3055 (2003).
25. J. W. Myatt, M. P. Healy, G. S. Bravi, A. Billinton, C. N. Johnson, K. L. Matthews, K. S. Jandu, W. Meng, A. Hersey, D. G. Livermore, C. B. Douault, J. Witherington, R. A. Bit, J. E. Rowedder, J. D. Brown, and N. M. Clayton, *Bioorg. Med. Chem. Lett.*, **20**, 4683 (2010).
26. Z. K. Sweeney, S. F. Harris, N. Arora, H. Javanbakht, Y. Li, J. Fretland, J. P. Davidson, J. R. Billedeau, S. K. Gleason, D. Hirschfeld, J. J. Kennedy-Smith, T. Mirzadegan, R. Roetz, M. Smith, S. Sperry, J. M. Suh, J. Wu, S. Tsing, A. G. Villaseñor, A. Paul, G. Su, G. Heilek, J. Q. Hang, A. S. Zhou, J. A. Jernelius, F.-J. Zhang, and K. Klumpp, *J. Med. Chem.*, **51**, 7449 (2008).
27. M. F. Braña, M. Cacho, M. L. García, E. P. Mayoral, B. López, B. de Pascual-Teresa, A. Ramos, N. Acero, F. Llinares, D. Muñoz-Mingarro, O. Lozach, and L. Meijer, *J. Med. Chem.*, **48***,* 6843 (2005).
28. A. Khodairy, *Synth. Commun.*, **31**, 2697 (2001).
29. A. Khodairy, *J. Chinese Chem. Soc.*, **54**, 93 (2007).
30. M. Abass and E. S. Othman, *Synth. Commun.*, **31**, 3361 (2001).
31. M. Abass, E. S. Othman, and A. Hassan, *Synth. Commun.*, **37**, 607 (2007).
32. M. Abass, *Phosphorus*, *Sulfur*, *Silicon*, *Relat. Elem.*, **182**, 735 (2007).
33. M. M. Ismail, *Chem. Papers*, **55**, 242 (2001).
34. A. Rahmati, *Tetrahedron Lett.*, **51**, 2967 (2010).
35. S. K. Panja, S. Maiti, S. Banerjee, and C. Bandyopadhyay, *Synlett* , 1909 (2010).
36. J. O. Subbotina, W. M. F. Fabian, E. V. Tarasov, N. N. Volkova, and V. A. Bakulev, *Eur. J. Org. Chem.*, 2914 (2005).
37. M. H. Elnagdi, F. M. Abdelrazek, N. S. Ibrahim, and A. W. Erian, *Tetrahedron*, **45**, 3597 (1989).
38. P. Trinder, *Ann. Clin. Biochem.*, **6**, 24 (1969).
39. G. Siest, J. Henny, and F. Schiele (editors), *Interprétation des examens de laboratoire. Valeurs de reference et variations biologiques*, Karger, Bâle, 1981, p. 206.