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Synthesis, and evaluation of α-amylase and α-glucosidase inhibitory potential of new pyrazolo[3,4-d]pyrimidine derivatives

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ABSTRACT

A series of new pyrazolo[3,4-d]pyrimidine compounds were synthesized in excellent yields via sulfuration and 1,3-dipolar cycloaddition and confirmed by MS, FT-IR and NMR techniques. All the prepared compounds were screened *in vitro* for their α-amylase and α-glucosidase inhibitory activities. Preliminary results indicated that some target compounds exhibited promising α-amylase and α-glucosidase inhibitory activity potency. Among the tested products, the cycloadduct f was found most active inhibitor (IC_{50} = 134.30 µM) for α-amylase, and the sulphur product b is the most active inhibitor (IC_{50} = 16.37 µM) for α-glucosidase.

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1. Introduction

Diabetes affects 415 million people worldwide and this number is projected to rise to 642 million by 2040 [1]. It is partly caused by the excessive presence of carbohydrates in the diet. Starch digestion in mammals is mainly carried out by α-amylase and α-glucosidase [2].

Type 2 diabetes mellitus (T2DM) is a complex and chronic metabolic disease due to insulin-resistance in body tissue [3]. The insulin resistance leads to hyperglycemia, which can damage many of organs [4]. One of the therapeutic approaches for alleviating T2DM is to suppress the glucose absorption from the intestine through the inhibition of carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase [5]. α-Amylase, which is mainly found in the saliva and pancreatic juice, breaks down large insoluble carbohydrate molecules into oligosaccharides, and α-glucosidase, which is in the brush border in the small intestine, is involved in the breakdown of disaccharides to glucose [6]. Inhibitors of α-amylase and α-glucosidase can delay the digestion of carbohydrates in the small intestine and reduce the level of postprandial blood glucose [7]. Many studies have searched for safe and effective inhibitors of α-amylase and α-glucosidase from medicinal plants to treat T2DM [8-11].

For our part, we study the inhibitory effect of α-amylase and α-glucosidase from chemical synthesis products derived of pyrazolo[3,4-d]pyrimidine. The pyrazolo pyrimidine ring is very interesting and versatile scaffold for the synthesis of potential drugs or molecular tools (Figure 1). The derivatives of pyrazolo[3,4-d]pyrimidine constitute class of heterocyclic compounds with interesting pharmacological properties [12-15]. Among their many applications, they are widely used for the treatment of gout [12], hyperuricemia [13], treatment and prevention of uric acid [14] and also for the treatment of primary or secondary symptomatic hyperuricemia [15].

In continuation of our work on the synthesis of the excess of allopurinol [16-23], we have envisioned the synthesis new pyrazolo[3,4-b]pyridine derivatives by thionation, alkylation and 1,3-dipolar cycloaddition reactions in order to evaluate their inhibitory effect of α-amylase and α-glucosidase.

2. Experimental

2.1. Instrumentation

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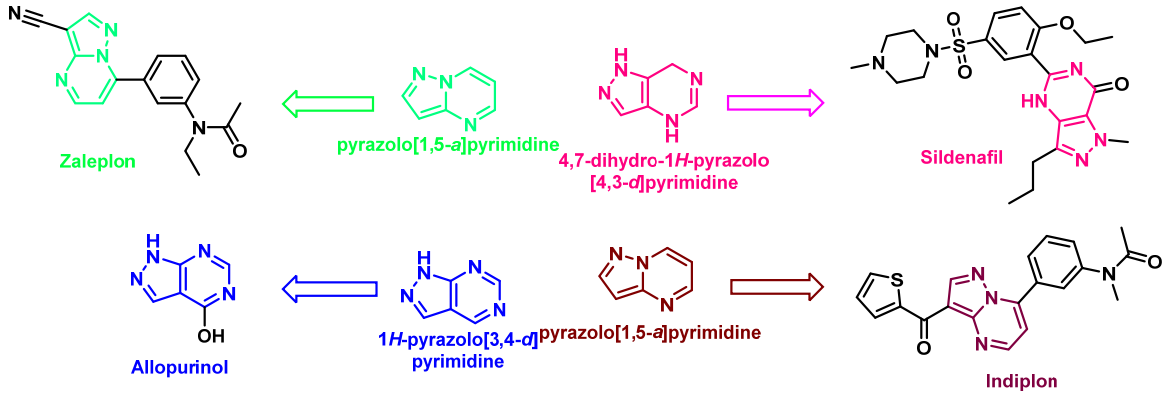


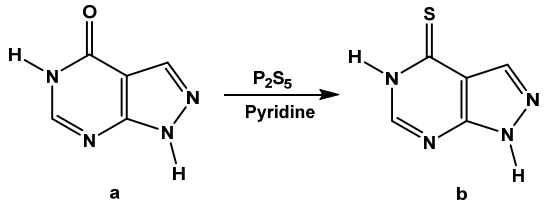
Figure 1. Pyrazolopyrimidine containing drugs.

The melting points were taken on an electrothermal capillary melting point apparatus. Infrared spectra were recorded on a Perkin Elmer 577, using KBr disks. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 300 NMR spectrometer in DMSO-d_6. Spectra were internally referenced to TMS. Peaks are reported in ppm downfield of TMS. Mass spectra are recorded in a Synapt G2 HDMS, Waters, Spectrometer in electrospray ionization (ESI). α-Amylase and α-glucosidase inhibitory activities were measured using 6705 UV/Vis Spectrophotometer (JENWAY).

2.2. Thionation of 1H-pyrazolo[3,4-d]pyrimidin4(5H)-one

The thionation reaction was carried out by the action of phosphorus pentasulfide on the pyrazolo[3,4-d]pyrimidine (**a**) in pyridine [9]. The reaction leads to pyrazolo[3,4-d]pyrimidine-4-thione (**b**), with yield of 90% (Scheme 1). Equimolar amounts of pyrazolo[3,4-d]pyridine (3.67 mmol) and phosphorus pentasulfide (3.67 mmol) in pyridine was heated to reflux for 4 hours. Then the solvent is evaporated under reduced pressure, the precipitate formed is washed with hot water to remove residual dimerized P_2S_5.

1H,4H,5H-pyrazolo[3,4-d]pyrimidine-4-thione (**b**): Color: Yellow. Yield: 90%. M.p.: 189-199 °C. FT-IR (KBr, ν, cm^{-1}): 1110 ν(C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 13.74 (s, 1H, NH), 13.13 (s, 1H, NH), 8.16 (s, 1H, =CH), 8.01 (s, 1H, =CH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 133.36 (=CH), 134.66 (=CH), 105.17, 146.84, 157.19 (Cq). MS (ESI, *m/z*): 153.0.



Scheme 1

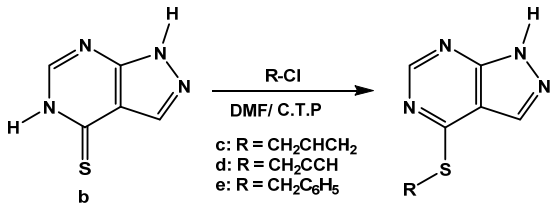
2.3. General procedure for the synthesis of(alkysulfanyl)-1H-pyrazolo[3,4-d]pyrimidine

1*H*,4*H*,5*H*-pyrazolo[3,4-d]pyrimidine-4-thione (3.29 mmol), alkyl bromide (5.70 mmol) and potassium carbonate (4.8 mmol) with a catalytic amount of tetra-*n*-butylammonium bromide were stirred in DMF (15 mL) for 48-72 h. The solid obtained was removed by filtration and the solvent evaporated under vacuum. The solid product was purified by recrystallization from ethanol (Scheme 2).

4-(Allylsulfanyl)-1H-pyrazolo[3,4-d]pyrimidine (**c**): Color: White. Yield: 60%. M.p.: 140-150 °C. FT-IR (KBr, ν, cm^{-1}): 1100 ν(C-S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 14.09 (s, 1H, NH), 8.72 (s, 1H, =CH), 8.27 (s, 1H,=CH), 4.02-4.09 (d, 2H, SCH_2, *J* = 2.48 Hz), 5.16-5.39 (d, 2H, =CH_2, *J* = 5.16 Hz), 5.94-6.02 (m, 1H, =CH, *J* = 5.98 Hz). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 15.11 (SCH_2), 132.62, 152.16 (=CH), 111.50, 154.49, 164.37 (Cq). MS (ESI, *m/z*): 193.1

4-(Prop-2-ynylsulfanyl)-1H-pyrazolo[3,4-d]pyrimidine (**d**): Color: White. Yield: 70%. M.p.: 130-140 °C. FT-IR (KBr, ν, cm^{-1}): 1100 ν(C-S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 13.98 (s, 1H, NH), 8.62 (s, 1H, =CH), 8.23 (s, 1H, =CH), 1.98 (s, 1H, C≡H), 4.03 (s, 2H, SCH_2). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 21.09 (SCH_2), 73.01 (HC≡), 133.03, 155.07 (=CH), 78.01, 112.415, 155.07, 162.39 (Cq). MS (ESI, *m/z*): 191.0.

4-Benzylsulfanyl-1H-pyrazolo[3,4-d]-pyrimidine (**e**): Color: Yellow. Yield: 70%. M.p.: 150-166 °C. FT-IR (KBr, ν, cm^{-1}): 1100 ν(C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 14.09 (s, 1H, NH), 8.76 (s, 1H, =CH), 8.25 (s, 1H, =CH), 7.20-7.45 (m, 5H, Ar-H), 4.65 (s, 2H, SCH_2). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 32.44 (SCH2), 132.60, 152.77 (=CH), 111.15, 127.78, 129.00, 129.50, 173.73, 152.77, 154.45, 163.94 (Cq). MS (ESI, *m/z*): 242.2.

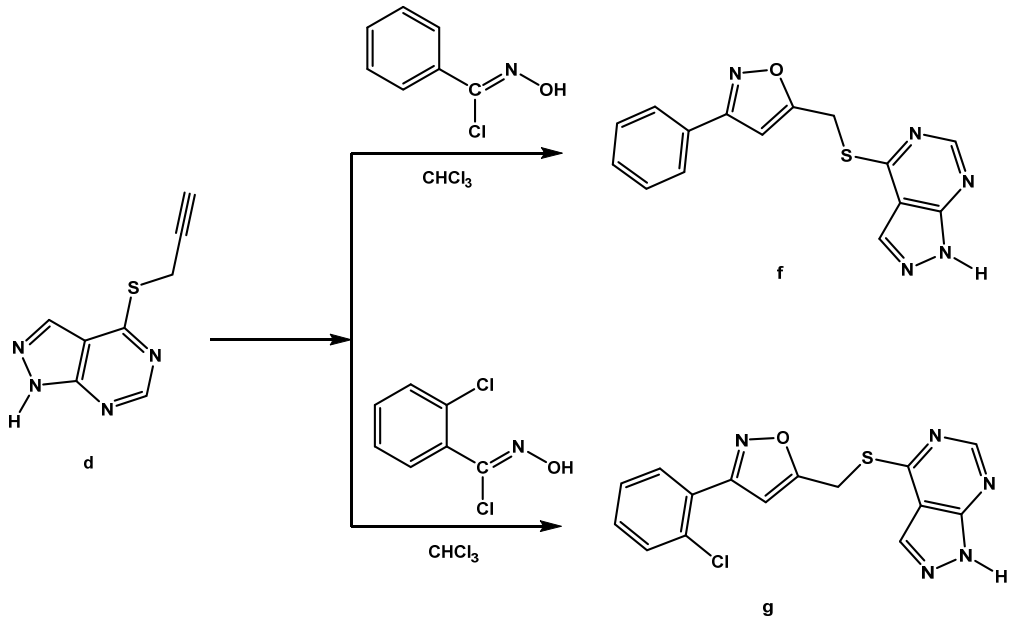


Scheme 2

2.4. General procedure for the synthesis of cycloadduct f and g

To a solution of 4-(prop-2-ynylsulfanyl)-1*H*-pyrazolo[3,4-d]pyrimidine (4.4 mmol) and 2-chlorobenzaldoxime /benzaldoxime (6.4 mmol) in 60 mL of chloroform was added 30 mL of liquid bleach with vigorous stirring for 2 hours. The organic phase obtained was dried over sodium sulphate, concentrated under reduced pressure. The residue obtained was chromatographed on silica gel (hexane:ethyl acetate, 80:20, *v:v*) (Scheme 3).

4-((3-Phenylisoxazol-5-yl)methylsulfanyl)-1H-pyrazolo[3,4-d]pyrimidine (**f**): Color: White. Yield: 40%. M.p.: 120-124 °C. FT-IR (KBr, ν, cm^{-1}): 1100 ν(C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 14.10 (s, 1H, NH), 8.09 (s, 1H, CH), 7.99 (s, 1H, CH), 6.83-7.97 (m, 5H, *J* = 7.7 Hz, Ar-H), 5.53 (s, 1H, CH), 4.52 (d, 2H, *J* = 7.2 Hz, SCH_2).



Scheme 3

^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 33.61 (CH_2), 100.80 ($CH_{isoxazolique}$), 99.70, 100.79, 117.03, 126.38, 133.23 (Cq), 129.26, 142.20 (CH_{Ar}), 143.87 (CH), 148.01 (CH). MS (ESI, *m/z*): 309.0.

4-((3-(2-Chlorophenyl)isoxazol-5-yl)methylsulfanyl)-1H-pyrazolo[3,4-d]pyrimidine (**g**): Color: Yellow. Yield: 40%. M.p: 110-120 °C. FT-IR (KBr, ν, cm^{-1}): 1100 ν(C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 14.06 (s, 1H, NH), 8.72 (s, 1H, CH), 8.71 (s, 1H, CH), 5.49-5.99 (m, *J* = 7.7 Hz, 4H, Ar-H), 5.30 (s, 1H, CH), 4.03 (d, 2H, *J* = 7.2 Hz, SCH_2). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 31.22 (CH_2), 111.39 (CH), 111.70, 118.86, 119.03, 125.37, 126.23 (Cq), 132.07, 133.67 (Ar-C), 154.29 (CH), 157.44 (CH). MS (ESI, *m/z*): 343.3.

2.5. In-vitro α-amylase and α-glucosidase inhibitory activity

2.5.1. α-Amylase inhibitory assay

α-Amylase inhibitory activity of the synthetic compounds was determined as previously described method [24]. A total of 250 µL of samples and 250 µL of 0.02 M sodium phosphate buffer (pH = 6.9) containing α-amylase solution (240 U/mL) were incubated at 37 °C for 20 minutes. After pre-incubation, 250 µL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH = 6.7) was added to each tube at timed intervals. The reaction mixtures were then incubated at 37 °C for 15 minutes. The reaction was stopped with 1.0 mL of dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 10 minutes, cooled to room temperature. The reaction mixture was diluted after adding 2 mL of distilled water and absorbance was recorded at 540 nm using the spectrophotometer.

2.5.2. α-Glucosidase inhibitory assay

α-Glucosidase inhibitory activity of the synthesized compounds was determined using the substrate pNPG according to the previously reported method [24]. A volume of 150 µL of sample solution and 100 µL of 0.1 M phosphate buffer (pH = 6.7) containing α-glucosidase solution (0.1 U/mL) was incubated at 37 °C for 10 minutes. After pre-incubation, 200 µL of 1 mM *p*-nitrophenyl-α-*D*-glucopyranoside solution in 0.1 M phosphate buffer (pH = 6.7) were added. The reaction mixtures were incubated at 37 °C for 30 minutes. After incubation, 1 mL of 0.1 M of Na_2CO_3 was added and the absorbance was measured at 405 nm in the spectrophotometer. The α-glucosidase and α-amylase inhibitory activity were expressed as percentage inhibition and the concentrations of tested compounds required to inhibit 50% of enzymes activity (IC_{50}) were determined. Acarbose was used as reference standard.

3. Results and discussion

3.1. Synthesis

Treatment of 1*H*-pyrazolo[3,4-d]pyrimidin-4(5*H*)-one (**a**) in absolute ethanol with phosphorus pentasulfide afforded the corresponding pyrazolo[3,4-d]pyrimidine-4-thione (**b**), with yield of 90% (Scheme 1). The structure of sulfur product **b** was elucidated by mass spectrum of the compound **b**, taken in electronic impact mode, indicates a molecular peak at *m/z* 152 [M+].

The action of one equivalent of the various alkylating agents (allyl chloride, propargyl chloride, benzyl chloride) on the pyrazolo [3,4-d] pyrimidine-4 (5*H*)-thione in DMF for 48 hours under conditions of the phase transfer catalysis (CTP) using BTBA as the catalyst and the carbonate potassium as a base [10,11], allows the exclusive alkylation of the sulfur atom and thus leads to formation of the S-alkylation product with a yield of 65% (Scheme 2). The structures of compounds **c**-**e** were determined by spectroscopic methods. The ^{1}H NMR spectra show, in particular, the signals relating to the alkyl groups, as well as the signals relating to the protons H_3 and H_6 of the bicyclic system. The ^{13}C NMR spectra show the signals relative to the carbons of the alkyl groups carried by the sulfur atom. An X-ray diffraction study confirmed the proposed structure, previously on the basis of the NMR spectra of compounds **c** and **e** [15,16].

1,3-Dipolar cycloaddition is a method of synthesis of five membered heterocycles, which are difficult to prepare by other means. Interestingly, condensation reaction of arylnitrile

oxide, prepared in situ by the action of water of Javel on benzaldehyde oxime, with propargylpyrazolo[3,4-d]pyrimidine **d** in a two-phase medium (water/chloroform) at 0 °C for 2 hours. It leads exclusively to cycloadducts **f** and **g** (Scheme 3). The structures of these cycloadducts were elucidated on the basis of the NMR spectral data, IR, and MS. The ^{1}H NMR of the compound f, essentially reveals the presence of a multiplet at δ 4.52 ppm, due to the SCH_2 group, and a signal at δ 6.53 ppm due to isoxazoline CH and ^{13}C NMR signal at δ 99.03 ppm. Examining the ^{1}H NMR of cycloadduct **g**, one notices in particular the appearance of multiplet at δ 4.03 ppm assigned to the SCH_2 group. Isoxazolinic CH appears as of a signal which resonates at δ 6.03 ppm and is manifested in ^{13}C NMR by the presence of a signal at δ 100.97 ppm. On the IR spectrum of pure isolated products, the presence of a strong absorption in the zone 1618-1646 cm^{-1} corresponding to the alkene function $\nu_{C=C}$ and $\nu_{C=N}$ for 1557cm^{-1}, at 1212 cm^{-1} corresponding to ν_{C-O} and ν_{C-N} around 841-936 cm^{-1}. These are the bands characteristic of the isoxazole ring.

3.2. In-vitro α-amylase and α-glucosidase inhibitory activity

The inhibitory activity of the synthesized compounds against α-amylase and α-glucosidase was measured spectrophotometrically. The results are shown in Table 1. Our compounds have showed moderateα-amylase and α-glucosidase inhibitory activities as compared to the reference compound acarbose which is a widely used anti-diabetic drug; product f was found most active inhibitor (IC_{50} = 134.30 µM) for α-amylase, and product **b** is the most active inhibitor (IC_{50} = 16.37 µM) for α-glucosidase. The inhibition of α-amylase and α-glucosidase activity in the digestive tract of humans is an important strategy in the management of postprandial blood glucose level in diabetic patients [25].

Table 1. α-Amylase and α-glucosidase inhibitory activity of the synthesized compounds.

Compound	IC_{50} (µM)	
	α-Amylase inhibition	α-Glucosidase inhibition
b	347.58±0.37	16.37±0.29
c	1336.72±0.39	893.52±0.08
d	259.40±0.17	178.91±0.30
e	986.25±0.41	690.90±0.57
f	134.30±0.39	89.63±0.66
g	515.80±0.37	360.10±0.11
Acarbose	482.02±1.38	27.89±2.00

The most commonly used pharmacological agents in the treatment of hyperglycemia such as acarbose is often associated with undesirable side effects especially abdominal distention, diarrhea, and flatulence [26,27]. The limitations of currently available drugs for the control of blood glucose have stimulated research on novel anti-hyperglecemic drugs. The results of our study may suggest these synthetic compounds as new molecular templates for development of novel agents for the management of postprandial hyperglycemia.

4. Conclusion

In conclusion, a series of pyrazolo[3,4-d]pyrimidine derivatives were synthesized successfully via thionation, alkylation and 1,3-dipolar cycloaddition reactions and cyclization. All these new compounds were confirmed by MS, FT-IR and NMR spectral techniques. Their α-amylase and α-glucosidase inhibitory activities were evaluated. The results showed that most of the synthesized pyrazolo[3,4-d]pyrimidine derivatives exhibited moderate inhibition of α-amylase and α-glucosidase activities, product **f** was found most active inhibitor (IC_{50} = 134.30 µM) for α-amylase, and product **b** is the most active inhibitor (IC_{50} = 16.37 µM) for α-glucosidase.

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