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In vitro biological studies and computational prediction-based analyses of pyrazolo[1,5-*a*] pyrimidine derivatives†

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There is a need for new pharmaceutical discoveries from bioactive nitrogenous derivatives due to the emergence of scourges, numerous pandemics, and diverse health problems. In this context, pyrazolo[1,5-*a*]pyrimidine derivatives **12a** and **12b** were synthesized and screened to evaluate their biological potentials *in vitro* as antioxidants, anti-diabetics, anti-Alzheimer's, anti-arthritics, and anti-cancer agents. Additionally, the computational pharmacokinetic and toxicity properties of the two pyrazolo[1,5-*a*]pyrimidines **12a** and **12b** were calculated and analyzed. The preliminary studies and results of this work represent the initial steps toward more advanced studies and define the bioactive chemical structure of pyrazolo[1,5-*a*]pyrimidine derivatives with the goal of exploring new drugs to address numerous health problems.

1. Introduction

Antioxidant agents are a category of synthetic or natural chemical substances that can scavenge, block, or reduce oxidative stress (high free radical concentration). The free radicals are produced by the body due to the normal use of oxygen. The free radicals cause cell damage and can lead to human diseases such as cardiovascular diseases, aging, diabetes, and cancer.[1,2] Examples of antioxidant agents include vitamin C (ascorbic acid), vitamin E (α-tocopherol), tripeptide glutathione (GSH), carotenoids, and flavonoids.[3]

Diabetes is a 21st-century challenge.[4] It is a deficiency in pancreatic function and secretion of the hormone insulin, which regulates glucose levels.[5] Contemporary studies refer to the relationship between obesity, diabetes, and its development.[6] The Arab world comprises 22 countries with 350 million humans. Six nations in the Arab world are on the top-ten list worldwide in the rate of diabetes and obesity majority. In general, almost 20% of the people in some Arab countries are diabetic.[7]

Pharmacological therapy for diabetes mellitus depends on the patient's case, in general, such as the influence of lifestyle modifications, during pregnancy, breastfeeding, or infection with other diseases.[8] The common class examples of anti-diabetic drugs are as follows: (I) sulfonylureas, there are two generations: tolbutamide represents the first generation while glipizide and gliclazide are representatives of the second generation. The action mechanism of sulfonylureas anti-diabetic drugs is to increase insulin secretion from pancreatic β cells.[9] (II) Alpha-glucosidase inhibitors (AGIs): there are various agents such as acarbose, miglitol, and voglibose, and the action mechanism is to decrease intestinal glucose absorption.[10] (III) Amylin analogs: pramlintide is an injectable amylin analog, and its action mechanism is to decrease glucagon release and slow gastric emptying.[11] Finally, (IV) biguanides: metformin is classified as a biguanides antidiabetic agent, and its action mechanism is to activate AMP-kinase and hepatic glucose production.[12]

Alzheimer's disease (AD) is a neurodegenerative disease that causes dementia.[13] In 2019, the studies conducted by El-Metwally and co-workers[14] demonstrated the following points: (I) Alzheimer's disease and dementia risk increased by various factors such as obesity, diabetes mellitus, and cardiovascular. (II) In Arab nations, dementia is a prevalent disease. (III) The statistical reports illustrated that between the age class of 50 and 80 years, dementia disease ranges from 1.1% to 2.3%, while in the age group of 80 years and older, dementia disease ranges from 13.5% to 18.5%. In the drug markets, there are various Alzheimer's agent therapies through acetylcholinesterase inhibition mechanism action; for example, galantamine is a type of phenanthrene alkaloid class, and donepezil is a piperidine

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class.[15] Additionally, Alzheimer's disease therapy through acetylcholinesterase and butyrylcholinesterase inhibition mechanism action, such as rivastigmine drug, is classified as a phenylcarbamate category.[16]

Cancer is a genetic disease caused (I) by errors that occur in cell division, (II) by DNA damage due to harmful substances, and (III) by inherited from parents. Therefore, the body's cells grow uncontrollably and sometimes spread to other parts; this is a cancer disease.[17] In 2020, the most prevalent cancers in Egypt are breast cancer and then liver cancer, as estimated by cancer statistics in Egypt. In addition, cancer mortality statistics refer to 89 042 cases for all cancer types (high mortality number).[18] In Saudi Arabia, the most common malignancies are breast and colorectal, and cancer mortality statistics refer to 12% cases for all types in 2016 but were approximately 5% in 1990.[19] As estimated by cancer statistics in the Arab World, in 2018, cancer was responsible for 16.1% of deaths in Tunisia,[20] while in 2016, it was responsible for 16.2% of deaths in Jordan.[21] Cancer treatment and care match the global trend, and cancer research is increasing in the Arab world.[22,23]

The therapy strategy for cancer depends on the organ affected, the rate of spread, and the patient's health state.[24] Cancer drug therapy is divided into classes according to its action mechanism as follows: (I) alkylating agents include

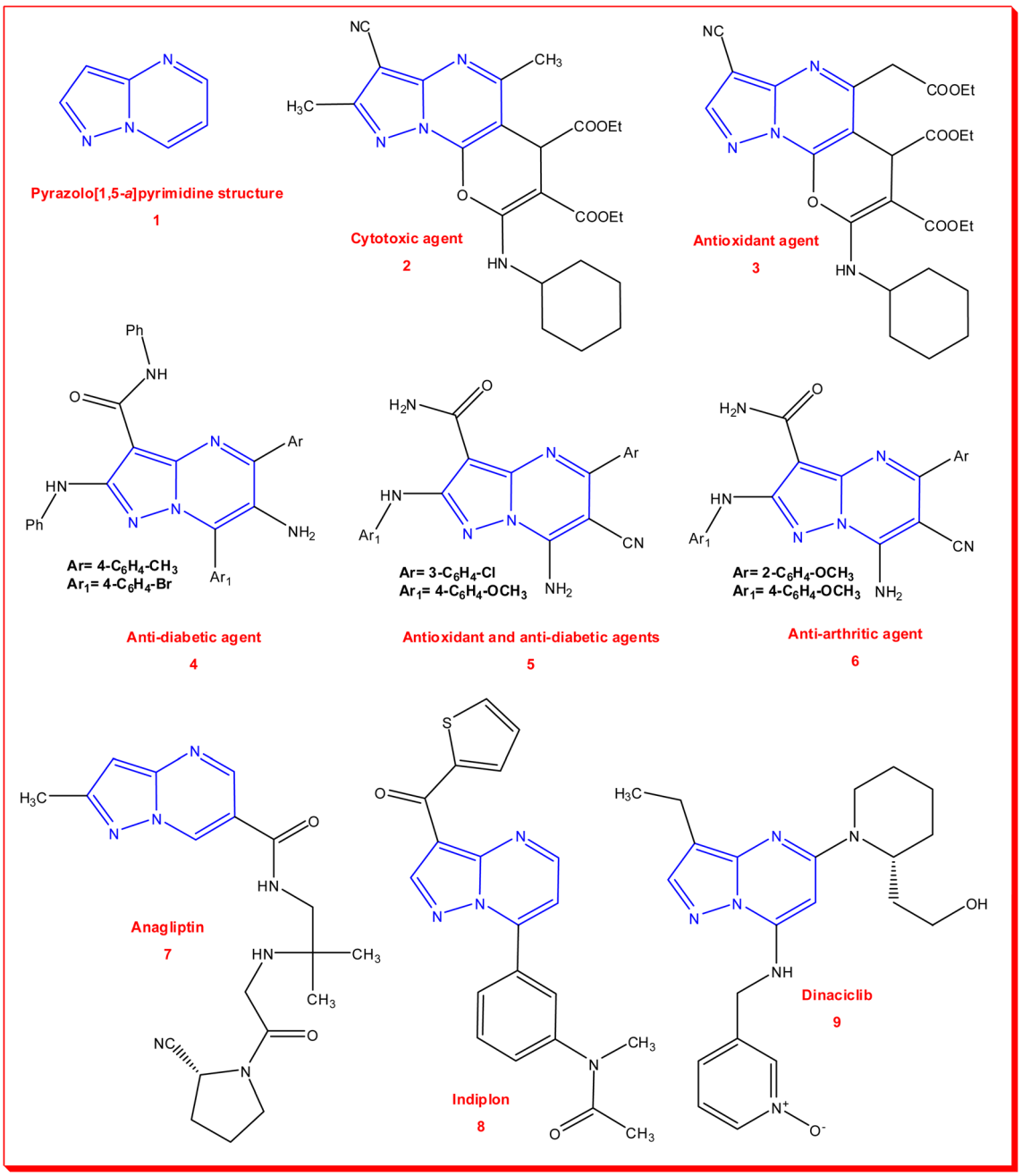


Fig. 1 Promising bioactivity of pyrazolo[1,5-*a*]pyrimidine derivatives **1–6** and its drug skeletons **7–9**.

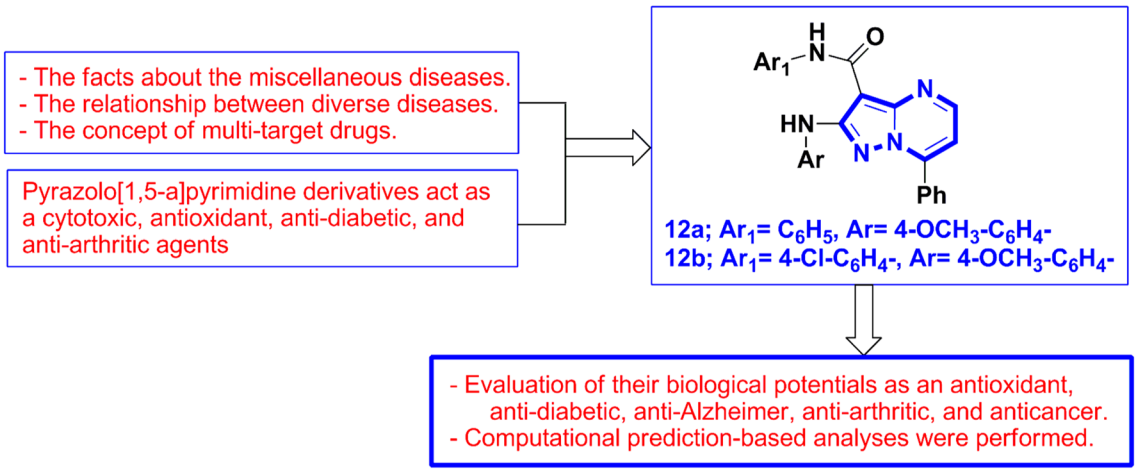


Fig. 2 Rationale and studies of pyrazolo[1,5-*a*]pyrimidine derivatives **12a** and **12b**.

temozolomide, cisplatin, and melphalan. These drugs add an alkyl group to the DNA of cells, damaging it; this mechanism functions at all phases of the cell cycle.[25] (II) Nitrosoureas include streptozocin and lomustine. These drugs possess the same action mechanism as alkylating agents but have the distinctive property that they can reach the brain.[26] (III) Antimetabolites include fluorouracil, decitabine, and thioguanine. These drugs interfere in DNA biosynthesis inducing in turn DNA replication inhibition.[27] (IV) Antitumor antibiotics include bleomycin, dactinomycin, doxorubicin, and epirubicin. These drugs modify the DNA in cells, inhibiting cancer's spread.[28] (V) Plant alkaloid topoisomerase inhibitors include mitoxantrone and teniposide; these drugs inhibit the separation of the two DNA strands through interaction with topoisomerase enzymes.[29] (VI) Mitotic inhibitors include paclitaxel and docetaxel; these drugs prevent cell mitosis.[30]

The literature has revealed, in the last decade, that pyrazolo[1,5-*a*]pyrimidine structure **1** has promising bioactivity in numerous pharmacological applications, such as anticancer,[31] antibacterial,[32] anti-COVID-19,[33] anti-HIV,[34] TNF-α inhibitor,[35] and anti-Alzheimer.[36] Additionally, Vahedi *et al.* synthesized 8-(cyclohexylamino)-2,5-dimethyl-6*H*-pyrano[3,2-*e*]pyrazolo[1,5-*a*]pyrimidine derivative **2**, which showed potent cytotoxicity against MCF-7 breast cells with IC_{50} = 19.70 ± 0.89 μM; they also prepared another 5-(2-ethoxy-2-oxoethyl)-6*H*-pyrano[3,2-*e*]pyrazolo[1,5-*a*]pyrimidinederivative **3** as an antioxidant agent, which exhibited free radical scavenging activity with IC_{50} = 12.12 ± 0.40 μM.[37] Peytam and co-workers prepared substituted 6-amino-pyrazolo[1,5-*a*]pyrimidine **4** as an anti-diabetic agent through the inhibition of α-glucosidase enzyme with an activity IC_{50} of 15.2 ± 0.4 μM, which is more potent than acarbose (IC_{50} = 750.0 ± 1.5 μM) by around 50-fold.[38] From our previous cooperation, Hassan *et al.* designed and prepared some 5-aryl-pyrazolo[1,5-*a*]pyrimidine derivatives as multi-target candidates and concluded that the 7-amino-6-cyano-pyrazolo[1,5-*a*]pyrimidine-3-carboxamide derivative **5** showed powerful activities as an antioxidant and anti-diabetic agent. They also recommended the 5-(2-methoxyphenyl)pyrazolo[1,5-*a*]pyrimidine derivative **6**, which presented anti-arthritic activity toward protein denaturation and proteinase with % = 20.66 ± 0.00 and 26.42 ± 0.06, respectively.[39] Furthermore, some marketed drugs possess pyrazolo[1,5-*a*]pyrimidine scaffolds in their structures for the treatment of miscellaneous diseases, such as anagliptin (**7**), which is a drug for type 2 diabetes mellitus treatment; indiplon (**8**), which is a sedative-hypnotic; and dinaciclib (**9**), a drug for cancer treatment through the inhibition of a cyclin-dependent kinase (CDK) enzyme[40,41] (Fig. 1).

Based on the scientific truths mentioned above about antioxidant agents, diabetes mellitus, Alzheimer's disease, cancer, disease statistics in the Arab world, and therapeutic strategies, the bioactive pyrazolo[1,5-*a*]pyrimidine and its drug derivatives as well as the continuation of our target of synthesizing bioactive nitrogenous derivatives,[42–44] in addition to the concept of multi-target drugs,[45–51] and the relationship between diverse diseases.[52–54] Accordingly, in this work, we selected the two pyrazolo[1,5-*a*]pyrimidines **12a** and **12b**, which possess pharmacology activities against MCF-7 and HepG-2 cell lines, respectively,[55] and possess promising antimicrobial and immunomodulatory activities[56] for evaluating of their biological potentials as antioxidant, anti-diabetic, anti-Alzheimer, anti-arthritic, and anticancer agents. Finally, computational prediction-based analyses were performed (Fig. 2).

2. Results and discussion

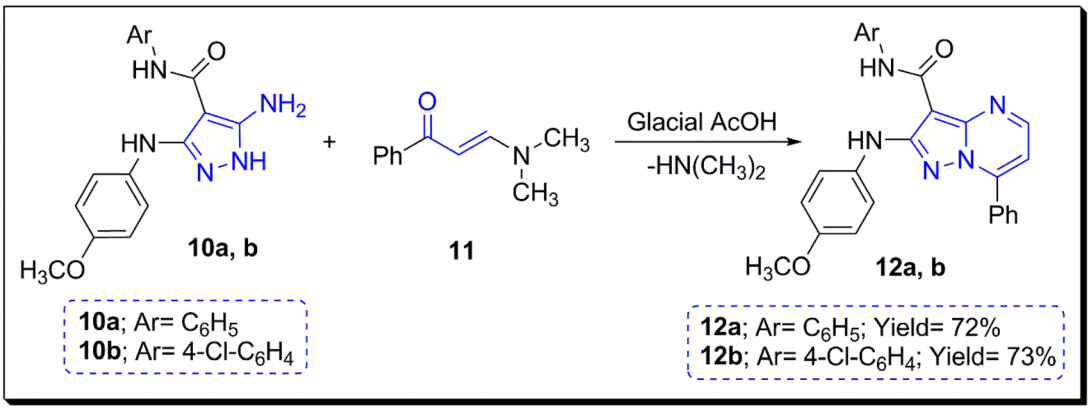
2.1. Chemistry

In this section, 5-amino-pyrazoles **10a** and **10b** benefited as starting materials for the preparation of the two selected pyrazolo[1,5-*a*]pyrimidines **12a** and **12b** *via* the direct condensation reaction with 3-(dimethylamino)-1-phenylprop-2-en-1-one (**11**) in AcOH as solvent[55] (Scheme 1).

The spectral data (^{1}H and ^{13}C NMR analyses) of pyrazolo[1,5-*a*]pyrimidines **12a** and **12b** are provided in the ESI.†

2.2. *In vitro* biological activities

2.2.1. Antioxidant activities of pyrazolo[1,5-*a*]pyrimidines 12a and 12b. The antioxidant activities of the two pyrazolo[1,5-



Scheme 1 Synthesis of pyrazolo[1,5-*a*]pyrimidines **12a** and **12b**.

Table 1 Antioxidant and scavenging activities of pyrazolo[1,5-*a*]pyrimidines **12a** and **12b**[a]

	Antioxidant activity		Scavenging activity	
Derivatives	TAC (mg gallic acid per g)	IRP (μg mL^{-1})	DPPH (IC_{50} μg ml^{-1})	ABTS (%)
12a	30.58 ± 0.07	17.29 ± 0.04	19.63 ± 0.04	25.28 ± 0.06
12b	31.27 ± 0.07	17.97 ± 0.04	18.33 ± 0.04	28.23 ± 0.06
STD	—	—	Ascorbic acid	
			4.05 ± 0.01	39.09 ± 0.09

[a] Values were calculated from three replicates and expressed as mean ± SE.

a]pyrimidine derivatives, **12a** and **12b**, were measured using the procedures described in ref. 57–60, and the results are depicted in Table 1.

Cancer, atherosclerosis, rheumatoid arthritis, and aging-related degenerative processes are prevalent diseases believed to involve excessive lipid oxidation and inflammation. The primary approach to preventing and treating these conditions may be to reduce these oxidation processes through the consumption of exogenous antioxidants.[61]

As shown in Table 1, it was observed that both the two pyrazolo[1,5-*a*]pyrimidines, **12a** and **12b**, had approximately the same total antioxidant capacity (TAC) and inhibitory radical potential (IRP). Numerically, *N*-(4-chlorophenyl)-pyrazolo[1,5-*a*] pyrimidine derivative **12b** exhibited slightly higher TAC (31.27 ± 0.07 mg gallic acid per g) and IRP (17.97 ± 0.04 μg mL^{-1}) compared to *N*-phenyl-pyrazolo[1,5-*a*]pyrimidine derivative **12a** (30.58 ± 0.07 mg gallic acid per g and 17.29 ± 0.04 μg mL^{-1}, respectively).

The antioxidant activity, as assessed by scavenging activities against DPPH and ABTS radicals, showed that both the two pyrazolo[1,5-*a*]pyrimidines, **12a** and **12b**, had nearly identical scavenging activities, but the numerical data supported the antioxidant activities. However, *N*-(4-chlorophenyl)-pyrazolo [1,5-*a*]pyrimidine derivative **12b** showed a lower IC_{50} value against DPPH (18.33 ± 0.04 μg mL^{-1}) and higher inhibitory activity against ABTS (28.23 ± 0.06%) at equal concentrations. The activity against the DPPH radical was expressed as IC_{50} values, with a low IC_{50} value indicating strong antioxidant activity. At the same concentration, the scavenging activity of the standard ascorbic acid against DPPH and ABTS was 4.05 ± 0.01 μg mL^{-1} and 39.09 ± 0.09%, respectively.

Similar redox properties enable the two derivatives of pyrazolo[1,5-*a*]pyrimidines, **12a** and **12b**, to act as hydrogen donors and reducing agents, which may be related to the similarity in their molecular structures.[62]

2.2.2. Anti-diabetic activities of pyrazolo[1,5-*a*]pyrimidines 12a and 12b

2.2.2.1. Enzyme assay. We measured the anti-diabetic activities of the two pyrazolo[1,5-*a*]pyrimidine derivatives, **12a** and **12b**, using the procedures from ref. 63–65. The results are shown in Table 2.

Diabetes mellitus (DM) is a chronic metabolic disease characterized by elevated glucose levels.[66] The enzymes α-amylase and α-glucosidase play a crucial role in regulating blood glucose levels, with α-amylase breaking down carbohydrates into disaccharides and α-glucosidase converting disaccharides into monosaccharides. Inhibiting these enzymes is a therapeutic strategy for controlling hyperglycemia.[67]

In the current study, *N*-(4-chlorophenyl)-pyrazolo[1,5-*a*] pyrimidine derivative **12b** exhibited the highest inhibitory effect on α-amylase (27.91 ± 0.02%), α-glucosidase (17.41 ± 0.02%), and β-glucosidase (9.66 ± 0.02%) compared to the standard acarbose, which had inhibitory effects at the same concentration on α-amylase (65.95 ± 0.01%), α-glucosidase (55.45 ± 0.01%), and β-glucosidase (47.70 ± 0.01%) (Table 2).

Table 2 Anti-diabetic activities of pyrazolo[1,5-*a*]pyrimidines **12a** and **12b**[a]

	α-Amylase		α-Glucosidase		β-Glucosidase	
Derivatives	Inhibition (%)	IC_{50} (mg ml^{-1})	Inhibition (%)	IC_{50} (mg ml^{-1})	Inhibition (%)	IC_{50} (mg ml^{-1})
12a	25.92 ± 0.01	1.92 ± 0.01	15.42 ± 0.01	3.13 ± 0.01	7.67 ± 0.01	6.47 ± 0.01
12b	27.91 ± 0.02	1.80 ± 0.01	17.41 ± 0.02	2.80 ± 0.01	9.66 ± 0.02	5.18 ± 0.01
	Acarbose					
Derivatives	Inhibition (%)	IC_{50} (mg ml^{-1})	Inhibition (%)	IC_{50} (mg ml^{-1})	Inhibition (%)	IC_{50} (mg ml^{-1})
STD	65.95 ± 0.01	0.76 ± 0.01	55.45 ± 0.01	0.90 ± 0.01	47.70 ± 0.01	1.07 ± 0.01

[a] Values were calculated from three replicates and expressed as mean ± SE.

The inhibitory activity is inversely proportional to the values of the IC_{50}, with lower IC_{50} values indicating higher inhibition. Compound **12b**, *N*-(4-chlorophenyl)-pyrazolo[1,5-*a*]pyrimidine derivative, had lower IC_{50} values against the activities of α-amylase (1.80 ± 0.01 mg mL^{-1}), α-glucosidase (2.80 ± 0.01 mg mL^{-1}), and β-glucosidase (5.18 ± 0.01 mg mL^{-1}) compared to the other tested *N*-phenyl-pyrazolo[1,5-*a*]pyrimidine derivative **12a**.

This may be attributed to the phenolic structure of the tested compounds, which are responsible for their inhibitory effect on these enzymes.[68] Hassan and Aboulthana suggested that these synthetic derivatives may belong to hypoglycemic substances, which could be caused by two different mechanisms: either by stimulating sugar-induced insulin secretion or by improving peripheral glucose intake.[50]

2.2.2.2. Native electrophoretic patterns. Electrophoresis is a widely used technique for separating, identifying, and quantifying different proteins and isoenzymes expressed in various tissues. It is commonly used to analyze the stoichiometry of a specific subunit of a protein complex.[69] Electrophoresis can detect mutagenic differences at a qualitative level by hiding normal bands and/or the appearance of abnormal ones. The significance index (SI) provides insight into the physiological state of the tissue and is inversely proportional to genetic variation, indicating qualitative alterations. Low SI values compared to the control group reveal differences in the number and arrangement of electrophoretically separated bands. Quantitative alterations, however, retain normal bands with their identification data, and changes occur in their quantities. Therefore, the SI value is not associated with quantitative changes.[70] α-Amylase, a digestive enzyme found mainly in saliva and pancreatic juice, catalyzes the hydrolysis of α-(1,4)-D-glycosidic linkages of starch and other glucose polymers, breaking down dietary carbohydrates into oligosaccharides and disaccharides. It is considered a potential target for diabetes.[71]

The electrophoretic α-amylase isoenzyme and α-glucosidase patterns of the two pyrazolo[1,5-*a*]pyrimidine derivatives, **12a** and **12b**, were assayed using the method suggested.[72,73] The results are illustrated in Fig. 3 and 4. Additionally, the main results of Native Electrophoretic Patterns are listed in Table S1 and S2 (ESI).†

The crude α-amylase enzyme was analyzed using electrophoresis, and two types were identified at Rfs 0.38 and 0.83 (Qty 10.33 and 7.32; B% 58.54 and 41.46, respectively) (Fig. 3 and Table S1†). This suggests its role in the metabolic pathway, as demonstrated by Aboulthana and co-workers.[74] When treated with pyrazolo[1,5-*a*]pyrimidine derivatives, **12a** and **12b**, at equal concentrations (1 mg mL^{-1}), the enzyme showed alterations, with one isoenzyme type (α-amy2) being hidden and an abnormal band appearing at Rf 0.68 with Qty 25.52 (B% 68.53) after treatment with *N*-phenyl-pyrazolo[1,5-*a*]pyrimidine derivative **12a** and Qty 9.27 (B% 50.50) after treatment with *N*-(4-chlorophenyl)-pyrazolo[1,5-*a*]pyrimidine derivative **12b**. Treatment with standard acarbose at the same concentration (1 mg mL^{-1}) caused severe abnormalities, hiding both types of the enzyme (α-amy1 and α-amy2) with an abnormal band identified at Rf 0.69 (Qty 9.94 and B% 100.00). Both pyrazolo[1,5-*a*]pyrimidine derivatives, **12a** and **12b**, caused physiological alterations in the α-amylase isoenzyme patterns when treated at equal concentrations (1 mg mL^{-1}), with the patterns being similar to the electrophoretic α-amylase isoenzyme pattern by the same percent (SI = 50.00%). When treated with concentrations equivalent to the IC_{50} values, *N*-phenyl-pyrazolo[1,5-*a*]pyrimidine derivative **12a** caused alterations by hiding one type of the enzyme (α-amy1) without changing the second one (α-amy2) identified at Rf 0.84 (Qty 23.87 and B% 100.00), while *N*-(4-chlorophenyl)-pyrazolo[1,5-*a*]pyrimidine derivative **12b** caused changes by hiding one type of the enzyme (α-amy2) without altering the second one (α-amy1) identified at Rf 0.37 (Qty 9.62 and B% 100.00). Both pyrazolo[1,5-*a*]pyrimidine derivatives, **12a** and **12b**, caused physiological alterations in the α-amylase isoenzyme patterns when treated at concentrations equivalent to values of the IC_{50}, with the patterns being similar to the electrophoretic α-amylase isoenzyme pattern by the same percent (SI = 33.33%). This study showed that pyrazolo[1,5-*a*]pyrimidine derivatives, **12a** and **12b**, caused qualitative alterations by changing the number and arrangement of the bands, hiding normal α-Amy type with or without an abnormal band, leading to lower values of the SI% compared to the crude enzyme. This is in agreement with Aboulthana *et al.*[75] who reported that alterations in the electrophoretic α-amylase isoenzyme pattern might be attributed to changing the fractional activity caused oxidatively by ROS. Abdel-Halim *et al.*[76] added that the structural changes induced in the protein portion of native enzymes by oxidative stress are responsible for changing the enzymatic activities of the α-amylase isoenzyme pattern.

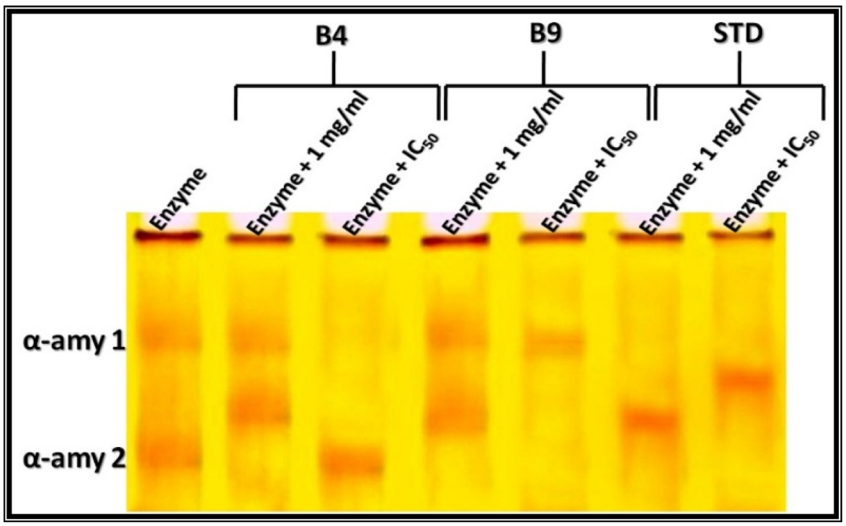


Fig. 3 Native electrophoretic α-amylase isoenzymes pattern showing the anti-diabetic activity of **12a** and **12b** derivatives compared to acarbose (standard) on the physiological state of α-amylase enzyme (B4 = **12a** and B9 = **12b**).

α-Glucosidase is a crucial enzyme in carbohydrate digestion, breaking down linear and branched isomaltose oligosaccharides to release glucose and causing postprandial hyperglycemia.[77] El-Shora and his team[78] visualized and confirmed the α-glucosidase using SDS-PAE, showing a single band with a molecular weight of 45 kDa.

In the current study, the crude α-glucosidase enzyme was identified as a single band at Rf 0.42 (Int. 115.00 and Qty 1.48). When treated with pyrazolo[1,5-*a*]pyrimidine derivatives, **12a** and **12b**, at equal concentrations (1 mg mL^{-1}), the band quantity decreased by 40.54% (Qty 0.88; Int. 100.80) with compound *N*-phenyl-pyrazolo[1,5-*a*]pyrimidine derivative **12a** and by 48.65% (Qty 0.76; Int. 87.25) with *N*-(4-chlorophenyl)-pyrazolo[1,5-*a*]pyrimidine derivative **12b**. Treatment with standard acarbose at the same concentration (1 mg mL^{-1}) caused a severe decrease in the band quantity by 84.14% (Qty 0.22; Int. 25.73). When treated with pyrazolo[1,5-*a*]pyrimidine derivatives, **12a** and **12b**, at concentrations equivalent to the IC_{50} values, the band quantity decreased by 56.08% (Qty 0.65; Int. 74.85) with compound *N*-phenyl-pyrazolo[1,5-*a*]pyrimidine **12a** and by 68.92% (Qty 0.46; Int. 52.89) with compound *N*-(4-chlorophenyl)-pyrazolo[1,5-*a*]pyrimidine **12b** (Fig. 4 and Table S2†). In contrast, treatment with standard acarbose caused severe alterations, completely decreasing the protein band. The electrophoretically detected alterations in the α-glucosidase pattern may be related to the disruption and denaturation of the α-glucosidase protein potentially due to the degree of ionization of certain amino acid side chains and/or the formation of

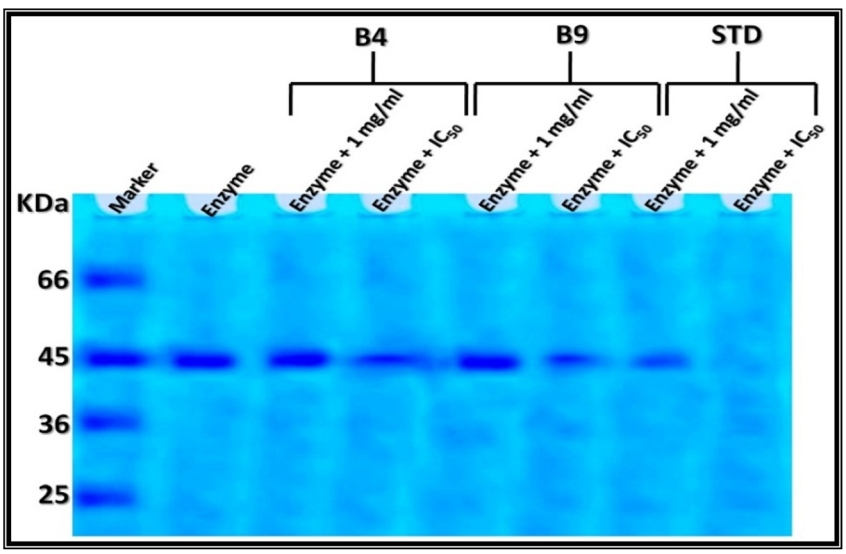


Fig. 4 Electrophoretic α-glucosidase enzyme checked by SDS-PAGE showing the anti-diabetic activity of **12a** and **12b** derivatives compared to acarbose (standard) on the physiological state of α-glucosidase enzyme (B4 = **12a** and B9 = **12b**).

reaction products or side-products that inhibit enzyme activity.[79]

2.2.3. Anti-Alzheimer's and anti-arthritic activities of pyrazolo[1,5-*a*]pyrimidines 12a and 12b. Alzheimer's disease is responsible for neurodegeneration, resulting in cognitive decline and death. Activation of the AChE enzyme is a leading cause of Alzheimer's disease. Therefore, inhibiting this enzyme is an effective treatment strategy for managing the disease.[80]

In the present study, we assessed the inhibition percentage of the acetylcholinesterase (AChE) enzyme using Ellman's method[81] and donepezil as the standard drug. It was observed that compound *N*-(4-chlorophenyl)-pyrazolo[1,5-*a*]pyrimidine **12b** exhibited the highest inhibitory effect on AChE activity (16.00 ± 0.04%), followed by compound *N*-phenyl-pyrazolo[1,5-*a*]pyrimidine **12a** (14.92 ± 0.02%) (Table 3). Compound *N*-(4-chlorophenyl)-pyrazolo[1,5-*a*]pyrimidine **12b** also had a lower IC_{50} value against AChE activity (3.15 ± 0.01 mg mL^{-1}) compared to compound *N*-phenyl-pyrazolo[1,5-*a*]pyrimidine **12a** (3.34 ± 0.01 mg mL^{-1}).

This finding is supported by Russo *et al.*[82] who suggested that compounds with antioxidant activities may also exhibit anti-diabetic and anti-Alzheimer properties. Therefore, derivatives with antioxidant activities could potentially be more effective in treating and managing diabetes and Alzheimer's disease. The standard drug donepezil exhibited an inhibitory activity of 71.14 ± 0.01% against AChE at the same concentration, with an IC_{50} value of 0.71 ± 0.00 mg mL^{-1}.

Arthritis is characterized by inflammation, which is one of its most important symptoms.[83] The ability of the tested compounds to inhibit proteinase denaturation and proteinase enzymes, which are key features and indices for the occurrence of inflammatory diseases, including arthritis, refers to the apparent potential for anti-inflammatory activity.[84] An important aspect of protein denaturation is the modification of forces that stabilize proteins essential for their structure and function, such as disulfide bridges, ionic interactions, electrostatic forces, and hydrogen bonds. Additionally, anti-inflammatory drugs inhibit protein denaturation in dose-dependent ways.[85]

The anti-arthritic activity was determined by quantifying the effectiveness of the pyrazolo[1,5-*a*]pyrimidines derivatives, **12a** and **12b**, in inhibiting protein denaturation and the activity of the proteinase enzyme using the procedures reported in previous works[86–88] and the results in Table 3.

The current study found that compound *N*-(4-chlorophenyl)-pyrazolo[1,5-*a*]pyrimidine **12b** had the highest inhibitory effect on proteinase denaturation (17.55 ± 0.04%) and proteinase activity (16.25 ± 0.04%), followed by compound *N*-phenyl-pyrazolo[1,5-*a*]pyrimidine **12a** (16.24 ± 0.04 and 14.91 ± 0.03%, respectively). The standard drug diclofenac sodium showed inhibitory activity of 49.33 ± 0.11% and 41.88 ± 0.09% against proteinase denaturation and proteinase activity, respectively, at the same concentration. The data on the antioxidant activities, anti-diabetic, anti-Alzheimer's and anti-arthritic activities of the tested derivative were positively correlated.[39,51] Additionally, *N*-(4-chlorophenyl)-pyrazolo[1,5-*a*]pyrimidine **12b** exhibited higher anti-arthritic activity due to the presence of 4-chlorophenyl rings, as suggested by Mezgebe and Mulugeta.[89]

2.2.4. Cytotoxicity. The two pyrazolo[1,5-*a*]pyrimidines, **12a** and **12b**, were examined *in vitro* for their cytotoxic activities (IC_{50}, μg mL^{-1}) against two human lung (A549) and colon (Caco-2) cancer cell lines, in addition to the normal lung (WI-38) cell line, and the results were compared with doxorubicin as a standard reference using the MTT assay.[90,91] It is well known that candidates that possess low IC_{50} and low side effects are considered promising cores for designing new cancer drugs.[92] The IC_{50} (μg mL^{-1}) values and corresponding therapeutic index (TI) of the two pyrazolo[1,5-*a*]pyrimidines, **12a** and **12b**, and doxorubicin are depicted in Table 4. Additionally, the main results of cytotoxic activities are listed in Table S3–S5, ESI.†

In the case of the A549 cell line, it was observed that compound **12b** exhibited slightly higher cytotoxic activity with a lower IC_{50} = 40.54 μg mL^{-1} compared to compound **12a** (IC_{50} = 47.83 μg mL^{-1}). However, both compounds showed lower cytotoxic activity compared to doxorubicin (IC_{50} = 31.32 μg mL^{-1}).

However, for the Caco-2 cell line, compound **12b** demonstrated higher cytotoxic activity with an IC_{50} of 29.77 μg mL^{-1}, which was relatively similar to the standard drug (IC_{50} = 28.45 μg mL^{-1}). Compound **12b** exhibits cytotoxic activity potentially due to its ability to induce G2/M arrest in cancer cells and increase the expression of tumor suppressor genes.

Table 3 Anti-Alzheimer's and anti-arthritic activities of pyrazolo[1,5-*a*]pyrimidines **12a** and **12b**[a]

	AChE		Anti-arthritic activity	
	Inhibition (%)	IC_{50} (mg mL^{-1})	Proteinase denaturation (%)	Inhibition of proteinase (%)
12a	14.92 ± 0.02	3.34 ± 0.01	16.24 ± 0.04	14.91 ± 0.03
12b	16.00 ± 0.04	3.15 ± 0.01	17.55 ± 0.04	16.25 ± 0.04
	Donepezil		Diclofenac sodium	
	Inhibition (%)	IC_{50} (mg mL^{-1})	Proteinase denaturation (%)	Inhibition of proteinase (%)
STD	71.14 ± 0.01	0.71 ± 0.00	49.33 ± 0.11	41.88 ± 0.09

[a] Values were calculated from three replicates and expressed as mean ± SE.

Table 4 *In vitro* cytotoxic activity values and corresponding therapeutic index of **12a**, **12b**, and doxorubicin against the two human cancer cell lines (lung (A549) and colon (Caco-2)) and the normal lung (WI-38) cell line

	IC_{50} (μg mL^{-1})			Therapeutic index (TI)	
Compounds	Lung cancer (A549)	Colon cancer (Caco-2)	Normal lung (WI-38)	Lung cancer (A549)	Colon cancer (Caco-2)
12a	47.83	38.15	134.24	2.8	3.5
12b	40.54	29.77	304.88	7.52	10.24
Doxorubicin	31.32	28.45	75.98	2.42	2.67

Additionally, it may bind not only with DNA but also with proteins in targeted cancer cells.[93] The cytotoxic activity may be linked to DNA interaction and cleavage, as well as the direct targeting of nucleic acids through the cleavage of DNA and RNA.[94] The newly synthesized compound induces oxidative stress, leading to cell death in cancer cells by increasing the total oxidant status and decreasing total antioxidant levels, thereby increasing oxidative stress levels.[95] The results suggest that compound **12b** demonstrates excellent cancer inhibition performance and could be considered a candidate drug for human lung and colon cancer types.

In the case of the normal lung (WI-38) cell line, it was observed that compound **12b** exhibited the lowest cytotoxicity, as evidenced by its highest IC_{50} value (304.88 μg mL^{-1}) compared to compound **12a** (134.24 μg mL^{-1}). Doxorubicin showed higher cytotoxicity on normal cells, with the lowest IC_{50} value of 75.98 μg mL^{-1}. These results suggest that compound **12b** is safer for normal cells than compound **12a**.

From this equation:

Therapeutic index (TI) = IC_{50} on the normal cells/IC_{50} on the cancer cells.

We can calculate the therapeutic index (TI) of the two pyrazolo[1,5-*a*]pyrimidines, **12a** and **12b**, to study their safety and efficacy.[96]

From Table 4, we can observe that the *N*-(4-chlorophenyl)-pyrazolo[1,5-*a*]pyrimidine derivative **12b** possesses a therapeutic index (TI = 7.52 and 10.24) higher than doxorubicin (TI = 2.42 and 2.67) in both cases of the A549 and Caco-2 lines, respectively.

3. Computational prediction

3.1. Pharmacokinetic prediction

The computational pharmacokinetic properties of the two pyrazolo[1,5-*a*]pyrimidines **12a** and **12b** were calculated using the free pkCSM website (**https://biosig.lab.uq.edu.au/pkcsm/**).[97] The results of the pharmacokinetic prediction properties are summarized in Table 5.

Cytochrome P450 (CYP) is a group of enzymes that metabolize drugs and other compounds. Five CYPs (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4) are responsible for metabolizing most approved drugs, and drug interactions involving CYPs can lead to premature termination of drug development and withdrawal from the market.[98] From Table 5, we found that the two pyrazolo[1,5-*a*]pyrimidines, **12a** and **12b**, are non-inhibitors of the CYP2D6 enzyme but inhibitors of other enzymes CYP1A2, CYP2C19, CYP2C9, and CYP3A4.

Table 5 Pharmacokinetic prediction properties of the two pyrazolo[1,5-*a*]pyrimidines **12a** and **12b**

Parameter	**12a**	**12b**
CYP1A2 inhibitor	Yes	Yes
CYP2C19 inhibitor	Yes	Yes
CYP2C9 inhibitor	Yes	Yes
CYP2D6 inhibitor	No	No
CYP3A4 inhibitior	Yes	Yes

3.2. Toxicity prediction

Computational toxicity estimations of the two pyrazolo[1,5-*a*]pyrimidines **12a** and **12b** were calculated using the free ProTox-II website (**https://tox-new.charite.de/protox_II/index.php?site=home**).[99] The four toxicity endpoints of the two pyrazolo[1,5-*a*]pyrimidines **12a** and **12b**, such as carcinogenicity, cytotoxicity, mutagenicity, and immunotoxicity, were predicted with a probability of more than 70%, which refers to the prediction's confidence estimate (confidence score). Additionally, the median lethal dose (LD_{50}, mg kg^{-1}) and toxicity class were predicted. The toxicity endpoint properties and the median lethal dose (LD_{50}) are summarized in Table 6.

Carcinogenicity is the ability to induce cancer. The substance may be active and cause cancer or is inactive and safe.[100] Based on computational estimation, *N*-(4-chlorophenyl)-pyrazolo[1,5-*a*]pyrimidine derivative **12b** is inactive, safe, and does not cause cancer, while *N*-phenyl-pyrazolo[1,5-*a*]pyrimidine derivative **12a** is active and causes cancer.

Cytotoxicity is the capacity of a substance to impact a cell, causing damage or death.[101] According to cytotoxicity prediction, the two pyrazolo[1,5-*a*]pyrimidines **12a** and **12b** are non-toxic to cells.

Mutagenicity is the ability to cause genetic mutations in DNA. The substance may be active, mutagenic, and cause genetic mutations, or inactive, non-mutagenic, and genetically safe.[102] Based on mutagenicity prediction, *N*-(4-chlorophenyl)-pyrazolo[1,5-*a*]pyrimidine derivative **12b** is inactive, non-mutagenic, genetically safe, and does not cause genetic mutations in DNA, while *N*-phenyl-pyrazolo[1,5-*a*]pyrimidine derivative **12a** is active, mutagenic, and causes genetic mutations in DNA.

Table 6 Toxicity endpoint properties and the median lethal dose (LD_{50}) of the two pyrazolo[1,5-*a*]pyrimidines **12a** and **12b**

Parameter	12a	12b
Toxicity endpoint properties		
Carcinogenicity	Active	Inactive
Cytotoxicity	Inactive	Inactive
Mutagenicity	Active	Inactive
Immunotoxicity	Inactive	Inactive
Median lethal dose (LD_{50}, mg kg^{-1})		
Predicted LD_{50}	1000	1000
Predicted toxicity class	4	4

Immunotoxicity refers to the adverse effects of substances on the immune system.[103] Based on this definition, the prediction table demonstrates that the two pyrazolo[1,5-*a*]pyrimidines **12a** and **12b** are safe for the immune system and do not cause adverse effects.

The median lethal dose (LD_{50}, mg kg^{-1}) is the dose that causes death in 50% of the animals tested. The toxicity classification of substances according to the Globally Harmonized System (GHS) is divided into six classes: class I ($LD_{50} \leq 5$) fatal if swallowed; class II ($5 < LD_{50} \leq 50$) fatal if swallowed; class III ($50 < LD_{50} \leq 300$) toxic if swallowed; class IV ($300 < LD_{50} \leq 2000$) harmful if swallowed; class V ($2000 < LD_{50} \leq 5000$) may be harmful if swallowed; and Class VI ($LD_{50} > 5000$) non-toxic.[104] According to the prediction study, the two pyrazolo[1,5-*a*] pyrimidines **12a** and **12b** possess a median lethal dose of LD_{50} that is equal to 1000 mg kg^{-1}. In this context, the two derivatives were classified as class IV.

4. Conclusions

In summary, we synthesized pyrazolo[1,5-*a*]pyrimidine derivatives **12a** and **12b**. Additionally, pyrazolo[1,5-*a*]pyrimidine derivatives **12a** and **12b** were screened to evaluate their biological potentials *in vitro*. The antioxidant results demonstrated that the *N*-(4-chlorophenyl)-pyrazolo[1,5-*a*]pyrimidine derivative **12b** exhibited slightly higher TAC (31.27 ± 0.07 mg gallic acid per g) and IRP (17.97 ± 0.04 μg mL^{-1}) compared to derivative **12a** (30.58 ± 0.07 mg gallic acid per g and 17.29 ± 0.04 μg mL^{-1}, respectively). Additionally, pyrazolo[1,5-*a*]pyrimidine derivative **12b** showed a lower IC_{50} value against DPPH (18.33 ± 0.04 μg mL^{-1}) and higher inhibitory activity against ABTS (28.23 ± 0.06%).

Anti-diabetic results refer to compound **12b** with lower IC_{50} values against the activities of α-amylase (1.80 ± 0.01 mg mL^{-1}), α-glucosidase (2.80 ± 0.01 mg mL^{-1}), and β-glucosidase (5.18 ± 0.01 mg mL^{-1}) compared to the other tested derivative **12a.** The electrophoretic isoenzyme pattern showed that the *N*-(4-chlorophenyl)-pyrazolo[1,5-*a*]pyrimidine derivative **12b** exhibited the highest anti-diabetic activity by altering the physiological state of α-amylase enzyme and denaturation of the protein portion in α-glucosidase enzyme. Pyrazolo[1,5-*a*]pyrimidine derivative **12b** exhibited the highest inhibitory effect on AChE activity (16.00 ± 0.04%) as an anti-Alzheimer's agent, and **12b** also had the highest inhibitory effect on proteinase denaturation (17.55 ± 0.04%) and proteinase activity (16.25 ± 0.04%) as an anti-arthritics agent.

In vitro cytotoxicity results indicate that pyrazolo[1,5-*a*]pyrimidine derivative **12b** possesses a lower IC_{50} of 40.54 and 29.77 μg mL^{-1} towards lung (A549) and colon (Caco-2), respectively. The safety and efficacy studies refer to derivative **12b** possessing a therapeutic index (TI) of 7.52 and 10.24, higher than doxorubicin (TI = 2.42 and 2.67) in both cases of the A549 and Caco-2 lines, respectively. According to the prediction study, the two pyrazolo [1,5-*a*]pyrimidines **12a** and **12b** are non-inhibitors of the CYP2D6 enzyme but inhibitors of other enzymes CYP1A2, CYP2C19, CYP2C9, and CYP3A4. In addition, the two derivatives **12a** and **12b** possess a median lethal dose of LD_{50}, which is equal to 1000 mg kg^{-1}. In this context, the two derivatives were classified as class IV. Pyrazolo[1,5-*a*]pyrimidine derivative **12b** is inactive and safe for carcinogenicity, cytotoxicity, mutagenicity, and immunotoxicity.

We are currently conducting additional mechanistic studies in our laboratories and will report our findings in the future.

5. Materials and methods

5.1. Chemistry

Pyrazolo[1,5-*a*]pyrimidines**12a** and **12b** were prepared according to the approach illustrated in our previous work, and their spectral data (^1H and ^{13}C NMR analyses) are presented in ESI†.[55]

5.2. *In vitro* biological activities

5.2.1. Antioxidant activity. The total antioxidant capacity (TAC) was measured in mg gallic acid/g by analyzing the green phosphate/Mo^{5+} complex at a wavelength (λ) of 695 nm, following the described procedure[57] (ESI†).

The iron-reducing power was determined in μg mL^{-1} using the method proposed by Oyaizu, with ascorbic acid as the standard[58] (ESI†).

The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activities were assessed using the method described by Rahman and Co-work[59] (ESI†).

For the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, the procedure followed the method suggested by Arnao *et al.* with some modifications[60] (ESI†).

5.2.2. Anti-diabetic activity

5.2.2.1. Enzyme assay. This assay involved calculating the inhibition percentage (%) of an α-amylase enzyme using the method based on the technique demonstrated by Wickramaratne (2016) with acarbose as the standard drug[63] (ESI†).

The inhibition percentage (%) of the α-glucosidase enzyme was determined using the method proposed by Pistia-Brueggeman and Hollingsworth with acarbose as the standard drug[64] (ESI†).

The β-glucosidase enzyme inhibition percentage (%) was measured using the *p*NPG method suggested by Han *et al.* with acarbose as the standard drug[65] (ESI†).

5.2.2.2 Native electrophoretic patterns

5.2.2.2.1 Electrophoretic α-amylase isoenzyme pattern. This assay used polyacrylamide gel electrophoresis (PAGE) following the method suggested by Rammesmayer and Praznik[72] (ESI†).

5.2.2.2.2 Electrophoretic α-glucosidase pattern. The vertical slab polyacrylamide gel electrophoresis (PAGE) was conducted following the method suggested by Laemmli using mini-gel electrophoresis (BioRad, USA) to determine the activity of the α-glucosidase enzyme[73] (ESI†).

5.2.3. Anti-Alzheimer's and anti-arthritic activities. In the anti-Alzheimer's activity study, we assessed the inhibition percentage of the acetylcholinesterase (AChE) enzyme using Ellman's method[81] and donepezil as the standard drug (ESI†).

In the anti-arthritic activity study, this assay involved determining the percentage of protein denaturation[86] and proteinase inhibition[87] using diclofenac sodium as the standard non-steroidal anti-inflammatory drug. The diclofenac sodium was prepared according to Meera *et al.*[88] (ESI†).

5.2.4. Cytotoxicity. The effectiveness of pyrazolo[1,5-*a*]pyrimidines **12a** and **12b** against human lung (A549) and colon cancer (Caco-2) cell lines, as well as the normal lung (WI-38) cell line, was determined by measuring the optical density (OD) at a wavelength of 590 using a 3-[4,5-dimethyl-2-thiazolyl]-2, 5-diphenyl-2*H*-tetrazolium bromide (MTT) assay.[90,91] The median inhibitory concentration (IC_{50}) calculation software was used to calculate the IC_{50} and percentage of cell growth inhibition (%) (ESI†).

Ethical statement

The experimental design involving human cancer cell lines was conducted in accordance with the protocol approved by the Medical Research Ethics Committee of the National Research Centre, located in Dokki, Cairo, Egypt (no: EX-11441223).

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1 V. Lobo, A. Patil, A. Phatak and N. Chandra, *Pharmacogn. Rev.*, 2010, **4**, 118–126.
2 K. Chand, A. Hiremathad, M. Singh, M. A. Santos and R. S. Keri, *Pharmacol. Rep.*, 2017, **69**, 281–295.
3 K. Jomova, R. Raptova, S. Y. Alomar, S. H. Alwasel, E. Nepovimova, K. Kuca and M. Valko, *Arch. Toxicol.*, 2023, **97**, 2499–2574.
4 P. Z. Zimmet, D. J. Magliano, W. H. Herman and J. E. Shaw, *Lancet Diabetes Endocrinol.*, 2014, **2**, 56–64.
5 M. S. Rahman, K. S. Hossain, S. Das, S. Kundu, E. O. Adegoke, M. A. Rahman, M. A. Hannan, M. J. Uddin and M. G. Pang, *Int. J. Mol. Sci.*, 2021, **22**, 6403.
6 L. Szczerbinski and J. C. Florez, *Lancet Diabetes Endocrinol.*, 2023, **11**, 861–878.
7 N. Alzaman and A. Ali, *J. Taibah Univ. Med. Sci.*, 2016, **11**, 301–309.
8 C. L. I. Rph and R. T. Tinong, *Cureus*, 2023, **15**, e44468.
9 A. Babiker and M. Al Dubayee, *Sudan. J. Paediatr.*, 2017, **17**, 11–20.
10 N. U. Khwaja and G. Arunagirinathan, *Curr. Drug Saf.*, 2021, **16**, 122–128.
11 M. Samsom, L. A. Szarka, M. Camilleri, A. Vella, A. R. Zinsmeister and R. A. Rizza, *Am. J. Physiol.: Gastrointest. Liver Physiol.*, 2000, **278**, G946–G951.
12 M. Foretz, B. Guigas, L. Bertrand, M. Pollak and B. Viollet, *Cell Metab.*, 2014, **20**, 953–966.
13 K.-Y. Yoo and S.-Y. Park, *Molecules*, 2012, **17**, 3524–3538.
14 A. El-Metwally, P. Toivola, M. Al-Rashidi, S. Nooruddin, M. Jawed, R. AlKanhal, H. A. Razzak and N. Albawardi, *Behav. Neurol.*, 2019, **2019**, 3935943.
15 G. T. Grossberg, *Curr. Ther. Res.*, 2003, **64**, 216–235.
16 S. Anwar, W. Rehman, R. Hussain, S. Khan, M. M. Alanazi, N. A. Alsaif, Y. Khan, S. Iqbal, A. Naz and M. A. Hashmi, *Pharmaceuticals*, 2023, **16**, 909.
17 H. S. Salem, *J. Cancer Res. Clin. Oncol.*, 2023, **149**, 5139–5163.
18 A. H. Ibrahim and E. Shash, General Oncology Care in Egypt, in *Cancer in the Arab World*, ed. H. O. Al-Shamsi, I. H. Abu-Gheida, F. Iqbal and A. Al-Awadhi, Springer, Singapore, 2022, 4, pp. 41–61, DOI: **10.1007/978-981-16-7945-2_4**.
19 M. A. Althubiti and M. M. NourEldein, *Saudi Med. J.*, 2018, **39**, 1259–1262.
20 N. Mejri, H. Rachdi, L. Kochbati and H. Boussen, General Oncology Care in Tunisia, in *Cancer in the Arab World*, ed H. O. Al-Shamsi, I. H. Abu-Gheida, F. Iqbal and A. Al-Awadhi, Springer, Singapore, 2022, 18, pp. 285–299, DOI: **10.1007/978-981-16-7945-2_18**.
21 S. Khatib and O. Nimri, General Oncology Care in Jordan, in *Cancer in the Arab World*, ed. H. O. Al-Shamsi, I. H. Abu-Gheida, F. Iqbal and A. Al-Awadhi, Springer, Singapore, 2022, 6, pp. 83–98, DOI: **10.1007/978-981-16-7945-2_6**.
22 A. A. Siddiqui, J. Amin, F. Alshammary, E. Afroze, S. Shaikh, H. A. Rathore and R. Khan, Burden of cancer in the Arab world, in *Handbook of Healthcare in the Arab World*, ed. I. Laher, Springer, Cham. 2021, 23, pp. 495–519. DOI: **10.1007/978-3-030-36811-1_182**.
23 R. R. Hamadeh, S. M. Borgan and A. M. Sibai, *Sultan Qaboos Univ. Med. J.*, 2017, **17**, e147–e154.
24 T. Schepis, S. S. De Lucia, A. Pellegrino, A. del Gaudio, R. Maresca, G. Coppola, M. F. Chiappetta, A. Gasbarrini, F. Franceschi, M. Candelli and E. C. Nista, *Cancers*, 2023, **15**, 3423.
25 R. Ralhan and J. Kaur, *Expert Opin. Ther. Pat.*, 2007, **17**, 1061–1075.
26 J. Fahrer and M. Christmann, *Int. J. Mol. Sci.*, 2023, **24**, 4684.
27 A. Lansiaux, *Bull. Cancer*, 2011, **98**, 1263–1274.
28 L. Xie, L. Cheng and Y. Wei, *Pathol., Res. Pract.*, 2023, **243**, 154350.

29 P. A. Yakkala, N. R. Penumallu, S. Shafi and A. Kamal, *Pharmaceuticals*, 2023, **16**, 1456.
30 M. Hashemi, M. A. Zandieh, Y. Talebi, P. Rahmanian, S. S. Shafiee, M. M. Nejad, R. Babaei, F. H. Sadi, R. Rajabi, Z. O. Abkenar and S. Rezaei, *Biomed. Pharmacother.*, 2023, **160**, 114392.
31 H. R. Elgiushy, S. H. Mohamed, H. Taha, H. Sawaf, Z. Hassan, N. A. Abou-Taleb, E. M. El-labbad, A. S. Hassan, K. A. M. Abouzid and S. F. Hammad, *Bioorg. Chem.*, 2022, **120**, 105646.
32 A. S. Hassan, G. O. Moustafa, A. A. Askar, A. M. Naglah and M. A. Al-Omar, *Synth. Commun.*, 2018, **48**, 2761–2772.
33 A. M. Abdelghany, T. K. Khatab and A. S. Hassan, *Bull. Chem. Soc. Ethiop.*, 2021, **35**, 185–196.
34 Y. Tian, D. Du, D. Rai, L. Wang, H. Liu, P. Zhan, E. De Clercq, C. Pannecouque and X. Liu, *Bioorg. Med. Chem.*, 2014, **22**, 2052–2059.
35 D. E. P. Rao, M. D. Raju, N. R. K. Reddy, C. Rajendiran, M. S. Praneeth, M. B. Tej, M. V. B. Rao, R. Kapavarapu and M. Pal, *Polycyclic Aromat. Compd.*, 2023, **43**, 1451–1468.
36 S. Kumari, K. Maddeboina, R. D. Bachu, S. H. Boddu, P. C. Trippier and A. K. Tiwari, *Drug Discovery Today*, 2022, **27**, 103322.
37 M. M. Vahedi, S. Asghari, M. Tajbakhsh, M. Mohseni and A. Khalilpour, *J. Mol. Struct.*, 2023, **1284**, 135446.
38 F. Peytam, M. Adib, R. Shourgeshty, L. Firoozpour, M. Rahmanian-Jazi, M. Jahani, S. Moghimi, K. Divsalar, M. A. Faramarzi, S. Mojtabavi and F. Safari, *Sci. Rep.*, 2020, **10**, 2595.
39 A. S. Hassan, N. M. Morsy, W. M. Aboulthana and A. Ragab, *Drug Dev. Res.*, 2023, **84**, 3–24.
40 S. Cherukupalli, R. Karpoormath, B. Chandrasekaran, G. A. Hampannavar, N. Thapliyal and V. N. Palakollu, *Eur. J. Med. Chem.*, 2017, **126**, 298–352.
41 C. Bagul, G. K. Rao, I. Veena, R. Kulkarni, J. R. Tamboli, R. Akunuri, S. P. Shaik, M. Pal-Bhadra and A. Kamal, *Mol. Diversity*, 2023, **27**, 1185–1202.
42 A. S. Hassan, S. A. Osman and T. S. Hafez, *Egypt. J. Chem.*, 2015, **58**, 113–139.
43 N. M. Morsy, A. S. Hassan, T. S. Hafez, M. R. H. Mahran, I. A. Sadawe and A. M. Gbaj, *J. Iran. Chem. Soc.*, 2021, **18**, 47–59.
44 S. S. Mukhtar, A. S. Hassan, N. M. Morsy, T. S. Hafez, F. M. Saleh and H. M. Hassaneen, *Synth. Commun.*, 2021, **51**, 1564–1580.
45 X. Chen and M. Decker, *Curr. Med. Chem.*, 2013, **20**, 1673–1685.
46 Q. Jiang, M. Li, H. Li and L. Chen, *Biomed. Pharmacother.*, 2022, **150**, 112974.
47 W. A. Badawi, M. Rashed, A. Nocentini, A. Bonardi, M. M. Abd-Alhaseeb, S. T. Al-Rashood, G. B. Veerakanellore, T. A. Majrashi, E. B. Elkaeed, B. Elgendy and P. Gratteri, *J. Enzyme Inhib. Med. Chem.*, 2023, **38**, 2201407.
48 H. N. Khedkar, L. C. Chen, Y. C. Kuo, A. T. Wu and H. S. Huang, *Int. J. Mol. Sci.*, 2023, **24**, 10247.
49 H. M. Alkahtani, A. A. Almehizia, M. A. Al-Omar, A. J. Obaidullah, A. A. Zen, A. S. Hassan and W. M. Aboulthana, *Molecules*, 2023, **28**, 7125.
50 A. S. Hassan and W. M. Aboulthana, *Egypt. J. Chem.*, 2023, **66**, 441–455.
51 A. S. Hassan, N. M. Morsy, W. M. Aboulthana and A. Ragab, *RSC Adv.*, 2023, **13**, 9281–9303.
52 I. Z. Sadiq, *Curr. Mol. Med.*, 2023, **23**, 13–35.
53 A. B. Jena, R. R. Samal, N. K. Bhol and A. K. Duttaroy, *Biomed. Pharmacother.*, 2023, **162**, 114606.
54 D. C. Shippy and T. K. Ulland, *Sci. Rep.*, 2023, **13**, 14800.
55 M. El-Naggar, A. S. Hassan, H. M. Awad and M. F. Mady, *Molecules*, 2018, **23**, 1249.
56 A. M. Naglah, A. A. Askar, A. S. Hassan, T. K. Khatab, M. A. Al-Omar and M. A. Bhat, *Molecules*, 2020, **25**, 1431.
57 P. Prieto, M. Pineda and M. Aguilar, *Anal. Biochem.*, 1999, **269**, 337–341.
58 M. Oyaizu, *Jpn. J. Nutr. Diet.*, 1986, **44**, 307–315.
59 M. M. Rahman, M. B. Islam, M. Biswas and A. H. M. Khurshid Alam, *BMC Res. Notes*, 2015, **8**, 621.
60 M. B. Arnao, A. Cano and M. Acosta, *Food Chem.*, 2001, **73**, 239–244.
61 Z. Kermanshah, H. Samadanifard, O. M. Moghaddam and A. Hejrati, *Pak. J. Med. Health Sci.*, 2020, **14**, 1301–1312.
62 W. M. K. Aboulthana, E. Refaat, S. E. Khaled, N. E. Ibrahim and A. M. Youssef, *Asian Pac. J. Cancer Prev.*, 2022, **23**, 3457–3471.
63 M. N. Wickramaratne, J. Punchihewa and D. Wickramaratne, *BMC Complementary Altern. Med.*, 2016, **16**, 466.
64 G. Pistia-Brueggeman and R. I. Hollingsworth, *Tetrahedron*, 2001, **57**, 8773–8778.
65 S. J. Han, Y. J. Yoo and H. S. Kang, *J. Biol. Chem.*, 1995, **270**, 26012–26019.
66 K. Kozieł and E. M. Urbanska, *Cells*, 2023, **12**, 460.
67 A. S. Alqahtani, S. Hidayathulla, M. T. Rehman, A. A. ElGamal, S. Al-Massarani, V. Razmovski-Naumovski, M. S. Alqahtani, R. A. El Dib and M. F. AlAjmi, *Biomolecules*, 2020, **10**, 61.
68 Z. D. Kifle, J. S. Yesuf and S. A. Atnafie, *J. Exp. Pharmacol.*, 2020, **12**, 151–167.
69 W. M. Aboulthana, M. Ismael and H. S. Farghaly, *Int. J. Curr. Pharm. Res.*, 2016, **7**, 347–359.
70 W. M. Aboulthana, W. G. Shousha, E. A.-R. Essawy, M. H. Saleh and A. H. Salama, *Asian Pac. J. Cancer Prev.*, 2021, **22**, 3267–3286.
71 M. Najafian, A. Ebrahim-Habibi, P. Yaghmaei, K. Parivar and B. Larijani, *Acta Biochim. Pol.*, 2010, **57**, 553–560.
72 G. Rammesmayer and W. Praznik, *J. Chromatogr. A*, 1992, **623**, 399–402.
73 U. K. Laemmli, *Nature*, 1970, **227**, 680–685.
74 W. M. Aboulthana, A. M. El-Feky, N. E. Ibrahim, R. K. Sahu and A. B. El-Sayed, *J. Appl. Pharm. Sci.*, 2018, **8**, 046–058.
75 W. M. Aboulthana, N. E. Ibrahim, A. K. Hassan, W. K. Bassaly, H. Abdel-Gawad, H. A. Taha and K. A. Ahmed, *J. Genet. Eng. Biotechnol.*, 2023, **21**, 158.

76 A. H. Abdel-Halim, A. A. Fyiad, W. M. Aboulthana, N. M. El-Sammad, A. M. Youssef and M. M. Ali, *J. Appl. Pharm. Sci.*, 2020, **10**, 077–091.
77 H. M. El-Shora, M. E. Ibrahim and M. W. Alfakharany, *Annu. Res. Rev. Biol.*, 2018, **24**, 1–9.
78 H. M. El-Shora, A. M. Metwally and A. K. Salwa, *Ann. Microbiol.*, 2009, **59**, 285–291.
79 H. El-Shora, S. M. Messgo, M. E. Ibrahim and M. W. Alfakharany, *Int. J. Phytomed.*, 2018, **10**, 175–180.
80 N. Suganthy, V. S. Ramkumar, A. Pugazhendhi, G. Benelli and G. Archunan, *Environ. Sci. Pollut. Res.*, 2018, **25**, 10418–10433.
81 G. L. Ellman, K. D. Courtney, V. J. Andres and R. M. Featherstone, *Biochem. Pharmacol.*, 1961, **7**, 88–95.
82 D. Russo, P. Valentão, P. Andrade, E. Fernandez and L. Milella, *Int. J. Mol. Sci.*, 2015, **16**, 17696–17718.
83 B. Balraj, N. Senthilkumar, I. V. Potheher and M. Arulmozhi, *Mater. Sci. Eng., B*, 2018, **231**, 121–127.
84 R. Ayman, A. M. Radwan, A. M. Elmetwally, Y. A. Ammar and A. Ragab, *Arch. Pharm.*, 2023, **356**, e2200395.
85 R. Thirumalaisamy, F. Ameen, A. Subramanian, T. Selvankumar, S. S. Alwakeel and M. Govarthanan, *Int. J. Pept. Res. Ther.*, 2010, **26**, 2179–2189.
86 S. Das and P. Sureshkumar, *Res. J. Biotechnol.*, 2016, **11**, 65–74.
87 O. O. Oyedapo and A. J. Famurewa, *Int. J. Pharmacogn.*, 1995, **33**, 65–69.
88 S. Meera, N. Ramaiah and N. Kalidindi, *Saudi Pharm. J.*, 2011, **19**, 279–284.
89 K. Mezgebe and E. Mulugeta, *RSC Adv.*, 2022, **12**, 25932–25946.
90 A. S. Hassan, H. M. Awad, A. A. Magd-El-Din and T. S. Hafez, *Med. Chem. Res.*, 2018, **27**, 915–927.
91 V. Vichai and K. Kirtikara, *Nat. Protoc.*, 2006, **1**, 1112–1116.
92 A. M. Hassan, A. O. Said, B. H. Heakal, A. Younis, W. M. Aboulthana and M. F. Mady, *ACS Omega*, 2022, **7**, 32418–32431.
93 J.-D. Hsu, S.-H. Kao, T.-T. Ou, Y.-J. Chen, Y.-J. Li and C.-J. Wang, *J. Agric. Food Chem.*, 2011, **59**, 1996–2003.
94 A. Barilli, C. Atzeri, I. Bassanetti, F. Ingoglia, V. Dall'Asta, O. Bussolati, M. Maffini, C. Mucchino and L. Marchiò, *Mol. Pharmaceutics*, 2014, **11**, 1151–1163.
95 Y. Wang, X. Zhang, Q. Zhang and Z. Yang, *BioMetals*, 2010, **23**, 265–273.
96 Y. Gorshkova, M.-E. Barbinta-Patrascu, G. Bokuchava, N. Badea, C. Ungureanu, A. Lazea-Stoyanova, M. Răileanu, M. Bacalum, V. Turchenko, A. Zhigunov and E. Juszynska-Galazka, *Nanomaterials*, 2021, **11**, 1811.
97 D. E. V. Pires, T. L. Blundell and D. B. Ascher, *J. Med. Chem.*, 2015, **58**, 4066–4072.
98 D. Ai, H. Cai, J. Wei, D. Zhao, Y. Chen and L. Wang, *Front. Pharmacol*, 2023, **14**, 1099093.
99 N. D. Rajaselvi, M. D. Jida, D. B. Nair, S. Sujith, N. Beegum and A. R. Nisha, *In Silico Pharmacol.*, 2023, **11**, 34.
100 D. C. Wolf, S. M. Cohen, A. R. Boobis, V. L. Dellarco, P. A. Fenner-Crisp, A. Moretto, T. P. Pastoor, R. S. Schoeny, J. G. Seed and J. E. Doe, *Regul. Toxicol. Pharmacol.*, 2019, **103**, 86–92.
101 O. A. Peters, *Int. Endod. J.*, 2013, **46**, 195–197.
102 K. Słoczyńska, B. Powroźnik, E. Pękala and A. M. Waszkielewicz, *J. Appl. Genet.*, 2014, **55**, 273–285.
103 M. B. Zerdan, S. Moussa, A. Atoui and H. I. Assi, *Int. J. Mol. Sci.*, 2021, **22**, 8242.
104 T. T. V. Tran, A. S. Wibowo, H. Tayara and K. T. Chong, *J. Chem. Inf. Model.*, 2023, **63**, 2628–2643.