Research article

Synthesis, anticancer activity and molecular docking of new quinolines, quinazolines and 1,2,4-triazoles with pyrido [2,3-*d*] pyrimidines

Ameen Ali Abu-Hashem [*], Othman Hakami, Nasser Amri

Department of Physical Sciences, Chemistry Division, College of Science, Jazan University, P.O. Box. 114, Jazan 45142, Kingdom of Saudi Arabia

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ABSTRACT

Recently, heterocyclic compounds such as pyrido [2,3-*d*] pyrimidinones, 1,2,4-triazolopyrimidines, pyrimidoquinazolines, and quinoline derivatives have gained attention from researchers due to their pharmacological and biological activities. To synthesize new compounds, quinoline-2-thioxopyrido [2,3-*d*] pyrimidinone (1) and methylthioquinoline-pyrido [2,3-*d*] pyrimidinones (2) were used as starting materials. The new compounds synthesized were quinoline-pyrido [2,3-*d*] (DeGoey et al., 2013; Gouda et al., 2020; Dangolani et al., 2018) [1, 2,4]triazolopyrimidinones (5a-d), 2-methylsulfonyl-quinoline-pyrido [2,3-*d*]pyrimidinone (6), pyrido [2,3-*d*]pyrimidine derivatives, pyridopyrimido (Gouda et al., 2020; DeGoey et al., 2013) 2,12,1-b] quinazoline (9), pyrido [(Khajouei et al., 2021; Gouda et al., 2020) 3,23,2-e]bis (1,2,4-triazole)pyrimidine (12a,b) and pyridopyrimido-diquinazoline-dione (16) derivatives. These compounds were synthesized with high efficiency, producing yields ranging from 69% to 90%, under moderate conditions, through treating (2) or (10) with various reagents such as anthranilic acid, phosphorus oxychloride, hydrazine hydrate, formic acid, glacial acetic acid, arylamine (aniline, 4-chloroaniline, or 4-methoxyaniline), and sec-amine (piperazine or morpholine). The new structures of the synthesized compounds were verified using various spectroscopic procedures, such as IR, NMR, and mass spectra. Molecular docking studies were carried out to investigate and discuss how the prepared compounds bind to amino acids such as Estrogen Receptor alpha, EGFR, and NADPH oxidase protein. Also, the synthesized products were tested for their anticancer and antioxidant activities against the (MCF-7) breast carcinoma cell line and human normal Retina pigmented epithelium cells (RPE-1). The study on the structure-activity relationship (SAR) established a correlation between the chemical structure of the newly synthesized compounds and their anticancer activity. The findings suggest that compounds 5a-d, 9,12a-b, and 16 exhibited promising anticancer activity and antioxidant effects as measured by DPPH inhibition.

1. Introduction

The human body comprises trillions of cells, encompassing various types such as skin, heart, liver, and muscle cells. These cells grow and divide as needed to maintain a healthy body. Cancer is an abnormal growth of cells in the body caused by a defect in the natural cellular systems that control cell division and reproduction. It can occur in any part of the body, leading to the old cells not

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dying and growing, forming abnormal cells. This results in the formation of masses of tissue known as tumors. Malignant tumors can spread to other body parts by transporting some cells from the tumor site to distant parts through the blood circulation or lymphatic system. When new tumors form in other body areas, this is known as the spread of cancer or metastasis. At this stage, cancer is considered advanced, which increases the risk and difficulty of treatment. Cancer treatment has been the focus of specialized doctors and researchers, who have explored several methods to treat this disease. One of the most used methods is chemotherapy. This treatment employs powerful chemicals that help eliminate rapidly growing cells in the body, making it a practical option for cancer treatment. Since cancer cells tend to grow and reproduce much quicker than other cells, chemotherapy is a preferred treatment method for many patients. Numerous chemotherapy drugs are available, each with unique properties and mechanisms of action, such as Fluorouracil **(I)**, Mercaptopurine **(II)**, Azacitidine **(III)**, Cytarabine **(IV)**, Floxuridine **(V)**, Doxorubicin **(VI)**, Thioguanine **(VII)**, Methotrexate **(VIII)**, Mitoxantrone **(IX)**, Valrubicin **(X)**, as shown in (Fig. 1).

We synthesized a range of new heterocyclic compounds during our research and assessed their anticancer properties. Previous studies have identified several moieties within these compounds that exhibit various biological activities. These activities can be shown as follows:

A literature survey revealed that pyrido [2,3-*d*] pyrimidine derivatives have important potential in pharmaceutical and medicinal chemistry due to their diverse range of biological activity, including anti-viral properties [1], antioxidant [2–5], antimicrobial [6–8], anti-inflammatory [9–11], antitubercular [12], it is also used as an inhibitor of some enzymes such as dihydrofolate reductase inhibition [13,14], glucosidase inhibition [15,16], a threonine tyrosine kinase (TTK) inhibition [17] and adenosine kinase inhibition [18]. The pyrimidine moiety has excellent chemical, physical and biological properties, especially when it is combined with heterocyclic compounds, for example: Thieno [2,3-d] pyrimidine is a compound that possesses antioxidant, anticancer, and antitumor properties [19–23]. Similarly, pyrazolopyrimidine, quinoxaline and pyrimidine are agents that have cytotoxic, anticancer, and antitumor properties [24–26]. Also, it has been found that pyrrolothiazolopyrimidines, triazolopyrrolothiazolopyramidines, and thiazolopyrimidines possess antioxidant and antitumor properties [27]. Similarly, pyrimidoquinazolines and azolopyrimidoquinolines are known for their antioxidants, analgesic, and antiinflammatory activities [28]. Additionally, pyrimido [4,5-*b*] quinolines exhibit several biological activities, such as antitumor, antioxidant, analgesic, and antiinflammatory activities [29–31]. On the other hand, the triazolopyrimidines and their structures of nucleoside analogues have various biological activities [32], medicinal and therapeutic influences, including antiparasitic [33], antimicrobial [34–37], antitumor [38–41], anticonvulsant [42], analgesic [42], anti-inflammatory [42,43], and antiviral [44–46] activities. Moreover, Pyridotriazolopyrimidinones, a fusion of pyridopyrimidine with triazole ring, possess important pharmacological and biological activities like antioxidant, antitumor, anti-HCV, antimicrobial, and anti-SSPE activities [47], anti-inflammatory, analgesic, antimicrobial, and antioxidant [48], anti-HCV and antitumor [49], antioxidant, active peroxynitrite inhibition and anti-HCV [50]. Furthermore, Triazolopyrimidines are purine isosteric similarities. They are minor molecule drugs that can affect protein metabolism and biosynthesis. For example, Trapidil **(XI)** is one such drug.
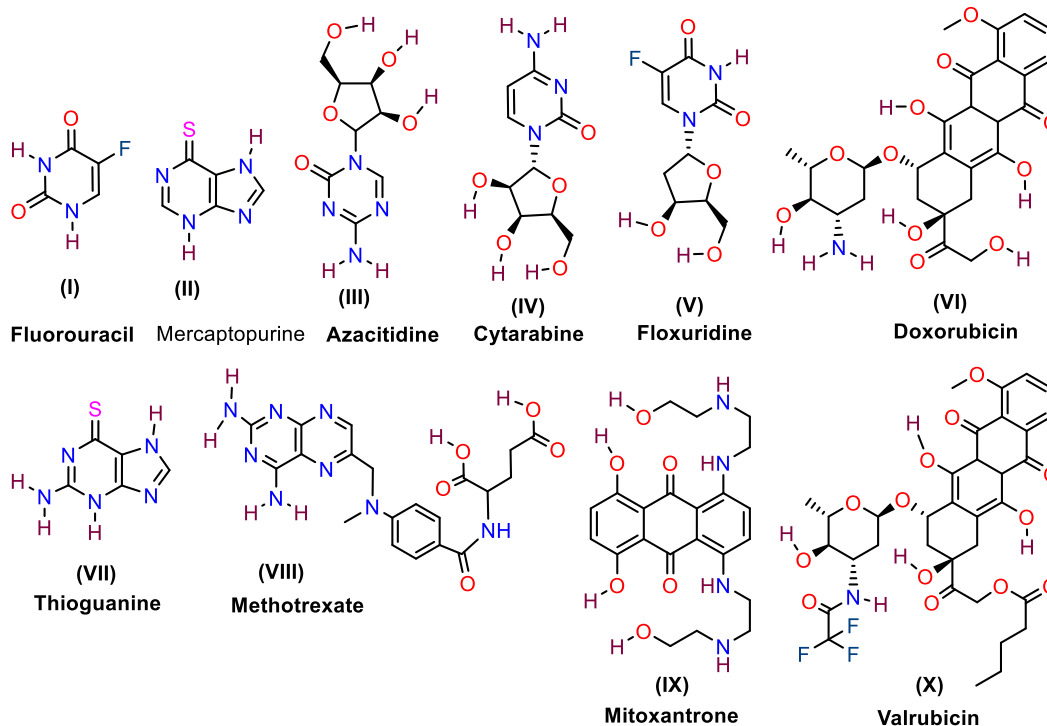

Fig. 1. Showed many drugs for the treatment of cancerous cells.

Triazolopyrimidines, such as triazolopyrimidine 2-sulfonamide, are used as herbicides in agrochemical applications. This compound is an analogue of the well-known agent Flumetsulam **(XII)**. The triazolopyrimidine (TP) molecule consists of two separate π-conjugated systems that self-assemble into super-molecular microfibres and exhibit blue-light releasing activities [08MOL855 **(XIII)**]. After investigating the absorption and spectral characteristics of styryl and triazolopyrimidines, substances like [09CNP101560211 **(XIV)**] were designed as electroluminescent materials with mechanochromic luminosity [51] shown in (Fig. 2).

Also, most natural compounds contain alkaloids, including quinoline derivatives with diverse biological activities. Researchers synthesized many quinoline derivatives, proving their biological and pharmacological activity, such as antihypertensive [52], antimicrobial [53,54], antileishmanial [55], antimalarial [56], antioxidant [57], anticancer [58], anti-asthmatic [59], antiviral [60], analgesic [61] and anti-inflammatory [62]. Also, Quinoline derivatives' small molecular size and hydrogen bonding ability make them useful in heterocycle preparations to obtain the structure of fluorescent chemicals [63,64].

Moxifloxacin [DB00218 **(XV)**] is a quinolone or fluoroquinolone antibiotic used to treat many microorganisms and bacterial infections (Aerobic Gram + Ve). It is marketed worldwide under the trademark name (Avelox). Lomefloxacin [DB00978 **(XVI)**] is a fluoroquinolone antibiotic used for bacterial infections including urinary tract infections and bronchitis, and to prevent UTIs before surgery. Mefloquine [DB00358 (XVII)] is a highly effective anti-malarial drug used to prevent and treat malaria caused by Plasmodium vivax and falciparum. Also, Amodiaquine [DB00613 **(XVIII)**] is a medication that possesses both antimalarial and anti-inflammatory properties. The drug comprises 4-aminoquinoline derivatives. Besides, Primaquine [DB01087**(XIX)**] is an antimalarial drug that is administered orally to prevent the recurrence of active malaria. Its chemical structure is aminoquinoline and it is used to achieve radical cure and avert the relapse of active malaria. As well as Carteolol drug [DB00521 **(XX)**] is a beta-adrenergic antagonist used to treat glaucoma, angina, arrhythmia, and hypertension. It is available in DrugBank online and shown in (Fig. 3).

In our previous articles, we have synthesized several classes of heterocyclic compounds and evaluated their pharmacological and biological activities [19–32,35–37,54].

So, this manuscript presents new pathways for synthesizing derivatives of quinoline-pyrido [2,3-*d*] pyrimidinones using various systems and approaches. These new compounds include pyrido [2,3-*d*] [1,2,4]triazolopyrimidinone; 2-methyl-sulfonyl-quinoline-pyridopyrimidinone; 2-(piperazin/morpholino)-5-(quinoline) pyrido [2,3-*d*]pyrimidinone; 2-((4-oxoquinoline)pyridopyrimidine)amino) benzoic acid; 4-quinoline-pyridopyrimido [2,1-*b*]qui-nazolines; 4-chloro-methylthio-quinoline-pyridopyrimidine; 2,4-dihydrazinyl (quinoline) pyridopyrimidine; substituted-12-quinoline-pyrido [3,2-*e*]bis ([1,2,4]triazolo) pyrimidine; N^2, N^4-sub-stituted-pyridopyrimidine-diamine; 7-phenyl-2,4-di (piperazin/morpholine)-5-(quinoline) pyridopyrimidine; 2,2'-((7-phenyl-5-(-quinoline) pyridopyrimidine) bis (azanediyl)) dibenzoic acid and 8-phenyl-6-(quinoline)-pyridopyrimido [2,1-*b*:4,3-*b*'] di-quinazoline-dione derivatives by utilizing various reagents. A group of new quinoline-pyridopyrimidine derivatives have been created and tested in laboratory conditions to assess their potential anti-proliferative properties on MCF-7 (breast adenocarcinoma cells) and RPE-1 (human normal Retina pigmented epithelium cells). Moreover, the derivatives have also been tested for their ability to prevent the activity of DPPH, which is an antioxidant.

2. Results and discussion

2.1. Synthesis

Abu-Hashem et al. have synthesized two compounds: substituted-quinoline-thioxopyrido [2,3-*d*] pyrimidinone **(1)** and methylthio-phenyl-quinoline-pyrido [2,3-*d*] pyrimidinones **(2)**, [54]. In this study, novel derivatives of quinoline-pyrido [2,3-*d*] [1,2,4] tri-azolopyrimidinones **(5a-d)** were obtained by reacting compound quinoline-thioxopyridopyrimidinones **(1)** with hydrazonoyl chloride derivatives **(3a-d)**. The reaction underwent a three-step process with intermediates **(4a-d), (4′a-d),** and **(4″a-d)**. The interaction during the cyclization process to form **(5a-d)** derivatives was found to be influenced *via* the nitrogen atom in position (*N*-3) rather than
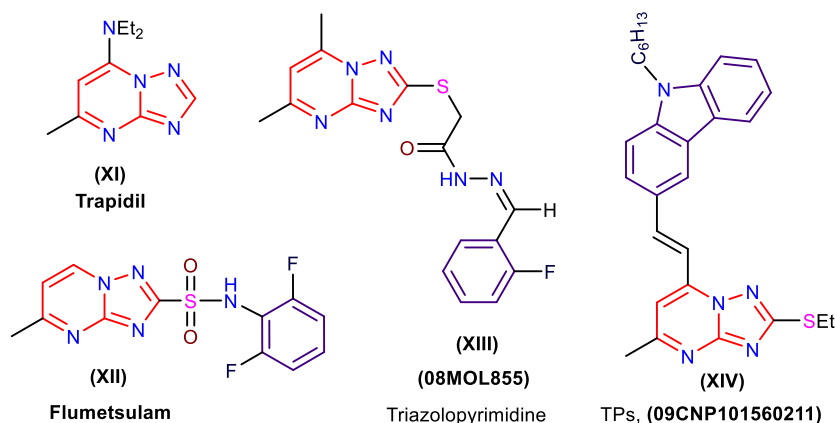

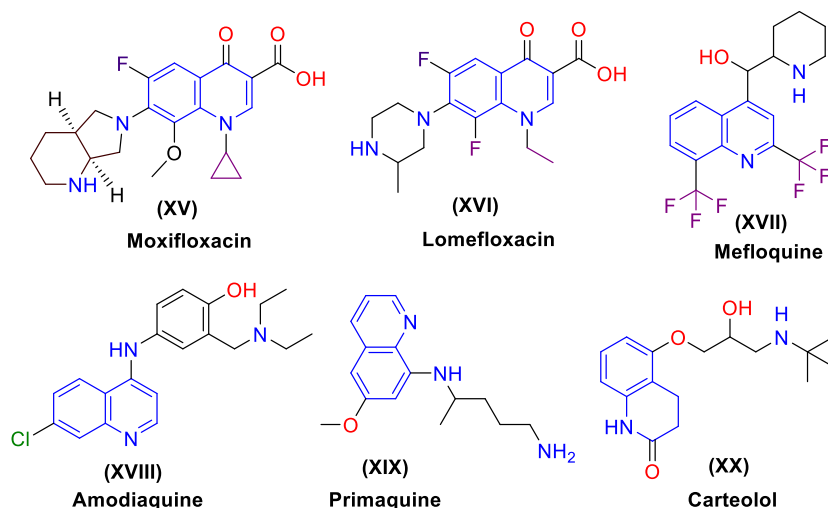**Fig. 2.** Showed many applications containing Triazolopyrimidines.

Fig. 3. Appeared of numerous drugs containing quinoline derivatives.

the nitrogen atom in position (*N*-1). The compound **(5a)** was analyzed using IR and ^{1}H NMR spectroscopy. The IR spectrum showed absorption bands at ν 3085 cm^{-1}, representing the existence of (CH) aromatic, and at 1685 cm^{-1}, confirming the existence of a carbonyl-amide group. The ^{1}H NMR spectra indicated the presence of three phenyl and quinoline rings, with twenty-one protons appearing as singlets at δ 7.10–7.93 ppm, and one proton of the pyridine ring appearing as one singlet at δ 8.05 ppm. The ^{13}C NMR (DMSO-d_6) spectra of **(5a)** had signals at δ 105.8 ppm (1C, CH, pyridine), 109.2, 110.5, 114.7116.5, 118.8, 120.4, 122.6, 126.5, 130.1, 131.7, 135.3, 137.5, 138.2, 139.4, 140.1, 140.7, 142.5, 146.8, 148.5, 151.2, 154.5, 155.6, 156.3, 157.9, 158.2, 161.1, 162.4 ppm (33C, Ar–C, sp^2 carbon atoms), and 173.5 ppm (1C, carbonyl group). The MS of **(5a-d)** established a molecular ion peak at *m/z*; 542 (M$^+$, 100%), 542 (M$^+$, 95%), 553 (M$^+$, 90%), and 538 (M$^+$, 85%), respectively.

All spectroscopic analyses, such as infrared (IR), nuclear magnetic resonance (NMR), mass spectrometry (MS), and elemental analyses, were fully described and characterized in the experimental section, as illustrated in (Scheme 1).

Likewise, 2-methylthio-phenyl-quinoline-pyrido [2,3-*d*] pyrimidinone **(2)** was oxidized with hydrogen peroxide in acetic acid to produce 2-methylsulfonyl-phenyl-quinoline-pyrido [2,3-*d*] pyrimidinone **(6)**. The infrared (IR) spectra of compound **(6)** revealed
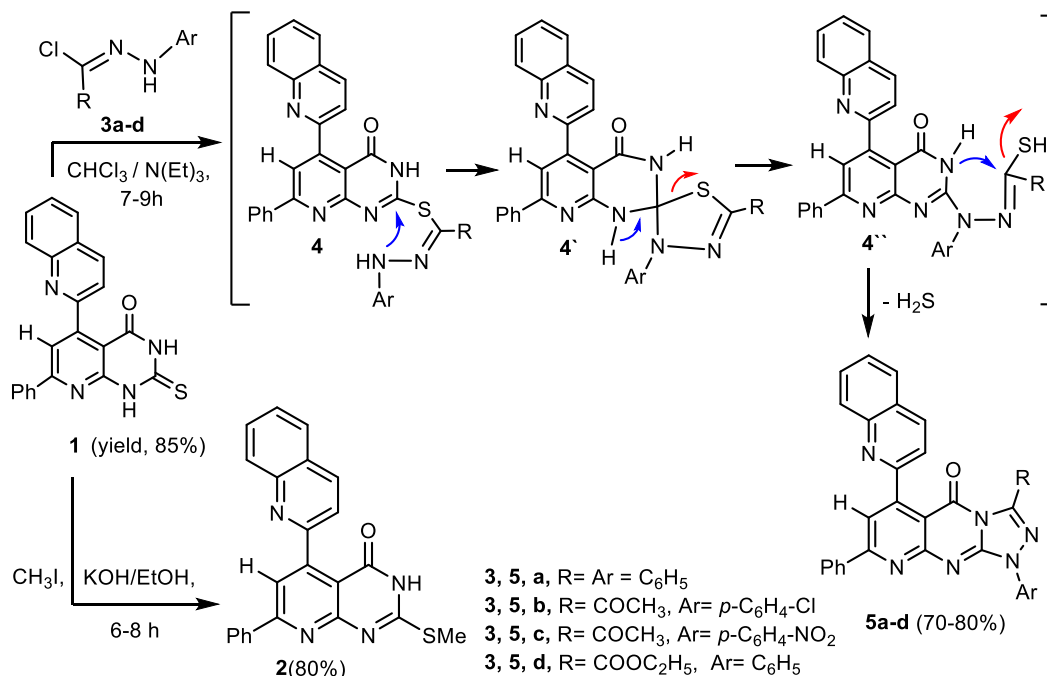
Scheme 1. preparation of quinolin-pyrido [2,3-*d*] [1,2,4] triazolopyrimidinone derivatives.

absorbed bands at ν 3250 cm^{-1}, indicating the presence of the (NH) group, and at 1677 cm^{-1}, confirming the amide carbonyl-amide group. In the ^{1}H NMR spectra of **(6)**, a singlet at δ 3.10 ppm was observed, corresponding to three protons of one (SO$_2$CH$_3$) group, and a singlet at δ 11.70 ppm was observed, representing the existence of one proton of the (NH) group, which is exchangeable with (D$_2$O). Treatment of 2-(methylthio) pyrido [2,3-*d*] pyrimidinone **(2)** with piperazine or morpholine in absolute methanol gave 2-piperazino- or 2-morpholino-phenyl-(quinoline) pyrido [2,3-*d*] pyrimidinones **(7a, b)**, respectively. A ^{1}H NMR spectrum of **(7a)** showed two singlets at δ 11.50 and 11.70 ppm matching the two protons of two groups (2NH), which are exchangeable *via* D$_2$O.

Also, reacting 2-methylthio-pyrido [2,3-*d*] pyrimidinone **(2)** with anthranilic acid in methanol produced 2-((oxo-phenyl (quinoline)pyrido [2,3-*d*] pyrimidine) amino) benzoic acid **(8)**. The infrared (IR) spectra of compound **(8)** display a broad band at ν 3510, 3380, and 3350 cm^{-1}, which belongs to three groups of hydroxyl groups (OH) and two groups of amino groups (2NH) respectively. Additionally, the spectrum exhibits sharp intensity peaks at ν 1722 and 1682 cm^{-1}, which correspond to two groups of carbonyls. The ^{1}H NMR spectrum of compound **(8)** reveals three singlet signals at δ 11.06, 11.52, and 11.73 ppm, which come from three groups of protons (2NH) and (OH), respectively, and the D$_2$O exchangeable evidence confirms the presence of these groups. The product **(8)** was cyclized *via* refluxing in (AcOH) with the addition of a few drops of (H$_2$SO$_4$) to provide 2-phenyl-4-(quinolin-2-yl)-5*H*-pyridopyrimido [2,1-*b*] quinazoline-dione **(9)**. A ^{1}H NMR spectrum of **(9)** displayed two singlet signals at δ 8.65 and 11.71 ppm, corresponding to one proton of pyridine and one proton of NH group with D$_2$O exchangeable, respectively. The compound 4-chloro-methylthio-phenyl-quinoline-pyrido [2,3-*d*] pyrimidine **(10)** was synthesized by refluxing 2-methylthiopyrido [2,3-*d*] pyrimidinone **(2)** with (POCl$_3$) in dry dioxane [28,30,65]. A ^{1}H NMR spectra of **(10)** showed two singlet signals at δ 1.25 ppm for three protons of (SCH$_3$) and 8.20 ppm for one proton of pyridine ring, respectively. Besides, the reaction and investigation of the latter compound **(10)** with hydrazine hydrate gave 2,4-dihydrazinyl-7-phenyl-5-(quinolin-2-yl) pyrido [2,3-*d*] pyrimidine **(11)**. Infrared (IR) spectrum of compound **(11)** showed absorption bands around the frequency range of ν 3440–3345 cm^{-1}, revealing the existence of two amine (2NH–NH$_2$) groups. The proton nuclear magnetic resonance (^{1}H NMR) spectrum of **(11)** showed two singlet signals at δ 6.70 and 6.80 ppm, conforming to the four protons of the (2NH$_2$) groups, which are exchangeable with deuterium oxide (D$_2$O), additionally, the spectrum showed two singlet signals at δ 10.90 and 11.80 ppm, conforming to the two protons of the (2NH) groups, which are also exchangeable with D$_2$O. Reaction of 2,4-dihydrazinyl-pyrido [2,3-*d*] pyrimidine **(11)** with (HCOOH or AcOH) yields new ring systems, 10-phenyl-12-(quinolin-2-yl)
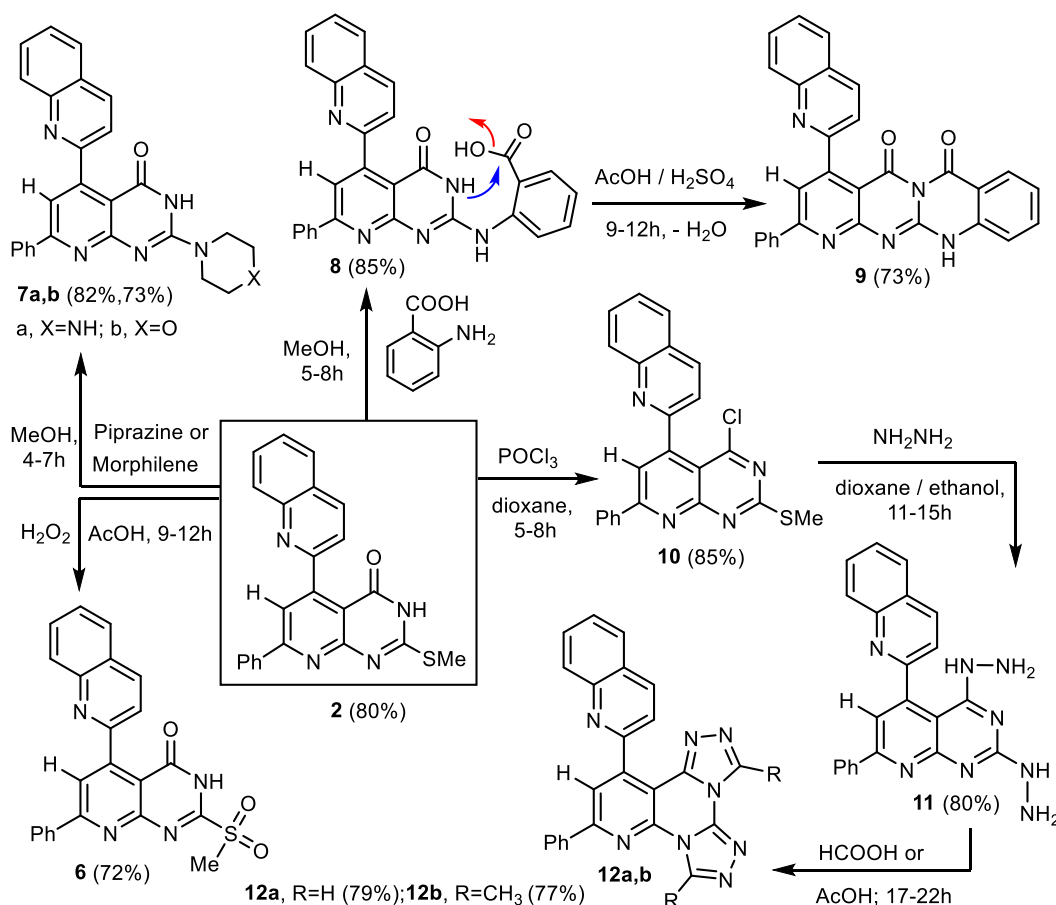
Scheme 2. Preparation of 4-(quinoline)-pyridopyrimido [2,1-*b*]quinazoline-dione and 12-quinoline-pyrido [3,2-*e*] bis (1,2,4-triazolo) [4,3-*a*:4′,3′-*c*] pyrimidine derivatives.

pyrido [3,2-e] bi s ([1,2,4] triazolo) [4,3-a: 4′, 3′-c] pyrimidine **(12a)** and **(12b)** respectively. The data obtained from the spectroscopic analysis of compounds **(12a, b)** supports the assigned structure. The IR spectrum of **(12a, b)** revealed the absence of (NH) and (NH$_2$) groups. The ^{1}H NMR spectrum of **(12a)** showed two singlet signals at δ 8.37 ppm for one proton of pyridine and δ 8.50, 8.75 ppm for two protons belonging to two (1,2,4-triazole) rings. The molecular ion peaks for compounds **(6, 7a, 7b, 8, 9, 10, 11, 12a and 12b)** were identified through mass spectrometry at m/z = 428 (M$^+$, 100%), 434 (M$^+$, 90%), 435 (M$^+$, 88%), 485 (M$^+$, 100%), 467 (M$^+$, 95%), 414 (M$^+$, 100%), 394 (M$^+$, 100%), 414 (M$^+$, 98%), and 442 (M$^+$, 92%), respectively. The experimental section displays the spectroscopy analysis results for the new compounds, as illustrated in (Scheme 2).

A 4-chloro-2-(methylthio)-5-(quinoline) pyrido [2,3-d] pyrimidine **(10)** underwent several nucleophilic substitutions, including primary and secondary aliphatic and aromatic amines like piperazine, morpholine and aryl amines. Refluxing compound **(10)** with 4-chloroaniline, p-anisidine, and the derivative of aniline in glacial acetic acid yields N^2, N^4-bis(substituted)-phenyl-quinoline-pyrido [2,3-d] pyrimidine-2,4-diamine **(13a-c)**. Infrared (IR) spectrum of **(13a-c)** showed absorbed bands at ν 3375-3352 cm^{-1} for 2NH groups. Furthermore, the ^{1}H NMR spectra of **(13a-c)** discovered the absence of a CH$_3$S group signal. The ^{1}H NMR spectrum of **(13a)** displayed three singlet signals at δ 8.08 ppm for one proton in pyridine and at δ 10.40 and 10.80 ppm for two protons in two (2NH) groups, which were found to be exchangeable with D$_2$O. Reaction of 4-chloro-2-(methylthio)-5-(quinoline) pyrido [2,3-d]pyrimidine **(10)** with sec-amine (20 mmol) such as piperazine and morpholine in glacial acetic acid produced two compounds: 7-phenyl-2,4-di (piperazin-1-yl)-5-(quinolin-2-yl) pyrido [2,3-d] pyrimidine **(14a)** and 4,4'-(phenyl-quinoline-pyrido [2,3-d] pyrimidine-2,4-diyl) dimorpholine **(14b)**. The ^{1}H- NMR spectrum of **(14a)** displayed a triplet at δ 1.70–1.80 and 2.10–2.20 ppm and another triplet at δ 2.70–2.80 and 3.75–3.85 ppm. These correspond to the sixteen protons of eight groups (CH$_2$) of two (piperazine) rings. Additionally, there were two singlets at δ 11.85 and 12.02 ppm, agreeing to two protons of two groups (2NH) via D$_2$O exchangeable. These findings support the suggested structure of the compound **(14a)**.
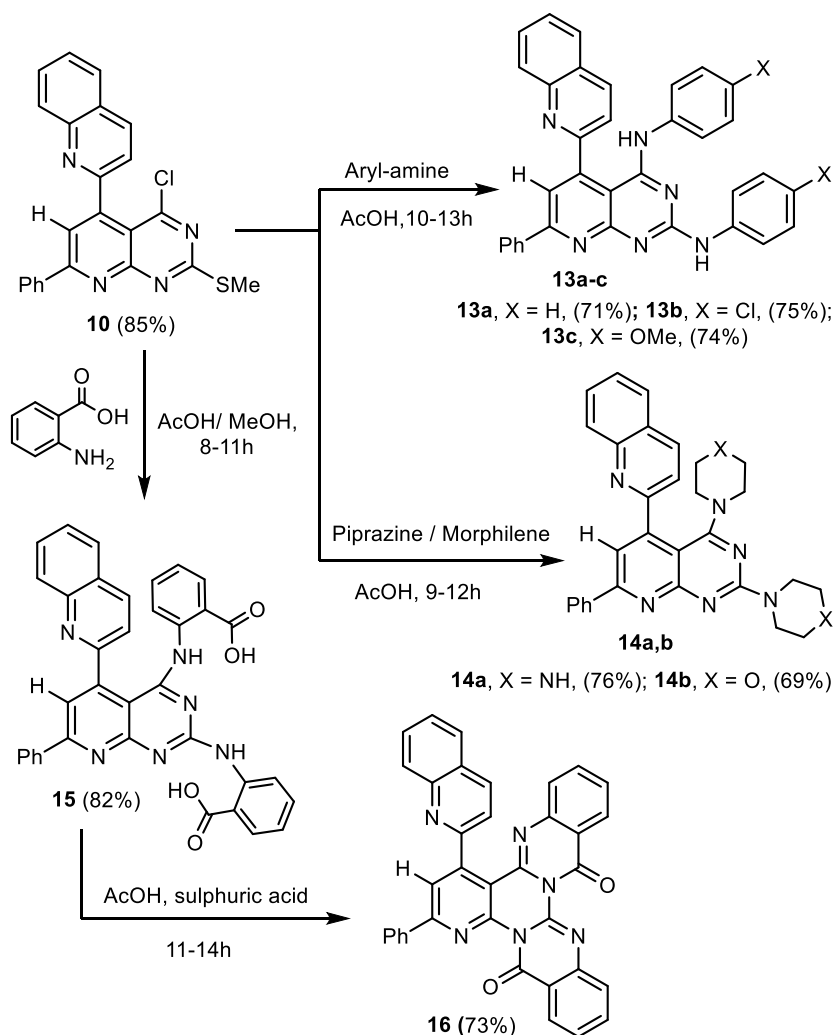
Scheme 3. Preparation derivatives of 8-phenyl-6-(quinoline)-pyridopyrimido [2,1-b:4,3-b'] diquinazoline-dione.

When 4-chloro-2-(methylthio)-5-quinoline-pyridopyrimidines **(10)** are treated with anthranilic acid in (MeOH/AcOH), it results in the formation of 2,2'- (phenyl-quinoline-pyridopyrimidine-2,4-diyl) bis (azane-diyl) dibenzoic acid **(15)**. The infrared (IR) spectra of **(15)** confirmed absorbed bands at around ν 3500-3480 cm^{-1} for two hydroxyl (OH) groups, soaked bands at ν 3380-3350 cm^{-1} for two amino (NH) groups, and absorbed bands at ν 1720, 1715 cm^{-1} to (2C═O) groups. The ^{1}H NMR spectra of **(15)** revealed four singlet signals at δ 11.54, 11.62 ppm for two protons of two (NH) groups, and 12.10, 12.85 ppm for two protons of two OH groups, which were confirmed by D$_2$O exchange. Moreover, the compound pyridopyrimidine-bis(azane-diyl) dibenzoic acid **(15)** was cyclized by heating and refluxing in acetic acid glacial with the existence of some drops of conc. sulfuric acid to yield 8-phenyl-6-(quinolin-2-yl)-11H,18H-pyridopyrimido [2,1-b: 4,3-b'] diquinazoline-11,18-dione **(16)**. The IR spectrum of **(16)** revealed that there were no absorbance bands in the (OH) and (NH) regions. It also showed a transfer of the carbonyl groups frequency from ν 1720, 1715 to 1690, 1685 cm^{-1}, which helped to establish the structure of the quinazoline ring. Additionally, the suggested format (see part experimental) was also proven *via* the ^{1}H NMR spectrum, as shown in (Scheme 3).

2.2. Biological activity

2.2.1. Anticancer activity

The newly prepared compounds underwent *in vitro* testing for anti-cancer and anti-proliferative properties against multiple human cancer cell lines, with a purity of 90–100%.

We conducted an evaluation on various compounds such as substituted pyrido [2,3-d] pyrimidinones, 1,2,4-triazolopyrimidines, pyrimidoquinazolines, and quinolines, to determine their cytotoxicity percentage (%) and antiproliferative activity against human cancer cell lines. The cell lines included MCF-7 breast adenocarcinoma cells and RPE-1 human normal Retina pigmented epithelium cells. We utilized the conventional MTT (3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) method to perform this assessment [66].

The study revealed that compounds **16, 12a-b, 9**, and **5a-d** exhibited significant antiproliferative activity versus (MCF-7 and RPE-1) carcinoma cell lines. The IC$_{50}$ values of these compounds ranged from 6.2 to 15.1 µM and 17.5–26.4 µM for MCF-7 and RPE-1 cell lines, individually. These compounds are sub-pyridopyrimido-diquinazoline-dione **(16),** sub-pyrido-bis ([1,2,4] triazolo) pyrimidine **(12a-b),** sub-pyridopyrimido [2,1-b] quinazoline-dione **(9)** and sub-pyridotriazolopyrimidinone **(5a-d)**. On the other hand, compounds **14a-b**, **13a-c** and **15** have adequate antiproliferative activity versus (MCF-7 and RPE-1) carcinoma cell lines. The IC$_{50}$ values of these compounds ranged from 18.7 to 27.1 µM and 28.1–38.2 µM for (MCF-7 and RPE-1) cell lines, respectively. The resting compounds exhibited weak activity versus the carcinoma cell lines. (Table 1).

2.2.2. Antioxidant activity

The antioxidant activities of the newly prepared compounds **(1-16)** were tested using DPPH assay and the results are presented in

Table 1

Shows the IC$_{50}$ values in µM for the antiproliferative activity of substituted-pyrid o [2,3-d], [1,2,4]triazolopyrimidine derivatives **(1-16)** versus some carcinoma cell lines, such as MCF-7 and RPE-1.

Compounds	IC50 (µM) ± SD		TI
	MCF-7	RPE-1	
1	45.5 ± 2.5	54.2 ± 2.3	2.4
2	43.2 ± 2.2	52.6 ± 1.9	2.2
5a	15.1 ± 2.3	26.4 ± 1.4	1.8
5b	10.2 ± 2.4	23.6 ± 1.5	1.9
5c	12.3 ± 2.4	24.1 ± 1.1	1.7
5d	13.5 ± 2.7	25.5 ± 1.8	2.2
6	37.8 ± 1.4	47.3 ± 1.7	1.6
7a	31.2 ± 1.8	42.1 ± 1.9	1.7
7b	32.5 ± 1.3	43.5 ± 1.5	1.4
8	35.4 ± 1.1	45.2 ± 1.5	1.1
9	8.1 ± 2.1	21.2 ± 1.1	1.6
10	40.1 ± 2.3	50.4 ± 1.8	2.1
11	29.5 ± 1.5	40.1 ± 1.6	1.5
12a	7.8 ± 1.1	20.9 ± 1.5	1.4
12b	7.5 ± 1.2	19.8 ± 1.4	1.3
13a	23.9 ± 2.1	34.7 ± 1.3	1.6
13b	21.8 ± 2.2	32.4 ± 1.7	1.9
13c	22.5 ± 2.3	33.2 ± 1.4	1.8
14a	18.7 ± 1.9	28.1 ± 1.6	1.7
14b	19.2 ± 1.5	29.3 ± 1.1	1.3
15	27.1 ± 1.2	38.2 ± 1.1	1.1
16	6.2 ± 1.3	17.5 ± 1.8	2.2
Doxorubicin	**10.5 ± 2.2**	**30.1 ± 3.1**	**2.1**
Sorafenib	**31.0**	——	——
lapatinib	**135.5**	——	——

Types of cells-breast adenocarcinoma cells (MCF-7) and human normal retina pigmented epithelium cells (RPE-1).

(Table 2).

2.2.3. Structural activity relationship (SAR)

2.2.3.1. In laboratory, antiproliferative activity (in vitro). After analyzing the data provided in (Table 1), the therapeutic index (TI) was calculated, which is the ratio of IC_{50} (50% inhibitory concentration) on normal cells to IC_{50} on cancer cells, we have concluded that products **16**, **12b**, **12a**, **9**, **5b**, **5c**, **5d**, and **5a**, in that order, are the most promising and effective potential anticancer drugs. These products have a significantly better TI in comparison to the standard drug, Doxorubicin.

2.2.3.2. Antioxidant activity. An assortment of derivatives, such as pyridopyrimidinones, 1,2,4-triazolopyrimidines, pyrimidoquinazolines, and quinolines, were tested for their antioxidant capacity **(Table 2)**, by inhibiting the "2,2-diphenyl-1-picrylhydrazyl" is a type of free radical (DPPH) [67,68]. Based on our results, we have found that all the compounds we tested exhibited moderate antioxidant activity in comparison to standard vitamins like ascorbic acid and rutin. However, it was instituted on the outcomes of both antioxidant and cytotoxicity tests, it seems unlikely that most of these new products can effectively scavenge the interactive oxygen radicals. Instead, most of these compounds possess radical compositions of the DPPH. The cytotoxicity activity of these compounds is high when they interact with human cancer and normal cells. Additionally, most compounds exhibit high cytotoxic effectiveness due to the rising intracellular oxidative exerted rate.

2.2.3.3. Study of structures activities relationship (SAR). Chemotherapy is a treatment that involves administering potent drugs to target and eliminate rapidly growing cells in the body. This treatment is commonly used to treat cancer as cancerous cells grow and multiply much faster than other cells in the body. There are various chemotherapy drugs available that can be used alone or in combination with other medications to treat different types of cancers. The manuscript's results highlighted the similarities between the chemical compounds of chemotherapy drugs and the newly synthesized and studied compounds. Specifically, these compounds' molecular structures and functional groups were compared, and the positive results obtained from the experiment were discussed based on previous studies and the structures activity relationship (SARs) and functional groups of these new compounds as follows: **(A)** The request for a wide-ranging of cytotoxicity activities may be due to the existence of different structures for new compounds such as quinoline, pyridopyrimidine, piperazine, morpholine, pyrido [2,3-*d*] [1,2,4] triazolopyrimidine; pyrido [2′,3′:4,5]pyrimido [2,1-*b*] quinazoline; pyrido [3,2-*e*]bis (1,2,4]triazolo)pyrimidine; N^2,N^4-bis(4-chlorophenyl); bis (azanediyl)) dibenzoic acid and pyridopyrimido-diquinazolines.

(B) The following compounds exhibited excellent efficacy against cancer cell lines *in vitro*: Substituted, -pyridopyrimido-diquinazoline-dione **(16)**, pyrido [3,2-e] bis ([1,2,4] triazolo) pyrimidines **(12a-b),** pyridopyrimido [2,1-*b*] quinazoline **(9)**, and pyrido-1,2,4-triazolopyrimidinone **(5a-d).** These results are consistent with previous scientific studies. Heterocyclic ring

Table 2
The compounds exhibited IC_{50} values for their DPPH inhibition activities.

Compounds	IC50 (μM) $\pm$ SD
1	188.8 $\pm$ 5.9
2	185.5 $\pm$ 7.2
5a	142.4 $\pm$ 4.8
5b	132.8 $\pm$ 5.1
5c	135.3 $\pm$ 4.5
5d	138.5 $\pm$ 5.9
6	178.4 $\pm$ 5.7
7a	171.6 $\pm$ 7.1
7b	174.5 $\pm$ 6.4
8	176.7 $\pm$ 5.2
9	127.2 $\pm$ 3.7
10	180.9 $\pm$ 4.7
11	168.5 $\pm$ 5.3
12a	121.1 $\pm$ 2.8
12b	120.4 $\pm$ 3.1
13a	161.9 $\pm$ 5.4
13b	155.1 $\pm$ 6.5
13c	158.5 $\pm$ 7.3
14a	146.6 $\pm$ 3.2
14b	148.9 $\pm$ 4.1
15	164.3 $\pm$ 4.9
16	123.5 $\pm$ 3.5
Rutin	**20.6 $\pm$ 2.1**
Vitamin C	**38.5 $\pm$ 2.4**

"2,2-diphenyl-1-picrylhydrazyl" is a type of free radical known as DPPH.

compounds have various pharmacological, biological, and optical activities. These compounds possess diverse properties, including cytotoxic activity, making them valuable in various fields. Examples of these compounds include quinoline, pyridine, pyrimidine, 1,2,4-triazole, quinazoline, 4-chlorophenyl, 4-methoxyphenyl, piperazine, and morpholine moieties. Some of these compounds contain functional groups such as methylthio, methylsulfonyl, and hydrazinyl, as well as heteroatoms like Nitrogen, Oxygen, and Sulfur. Previous research and studies have confirmed that some of these compounds possess high biological activity. For example, triazolotetrahydropyrimidoquinoline and triazolopyrimidoquinoline display potent antitumor activity [29, 69]. Further, pyrimidine derivatives, such as thienopyrimidine, thienopyrazole, and thiophene-carbohydrazide, have been studied for their potential as antioxidants and antitumor agents [22,23]. Besides, some polycyclic aromatic compounds such as pyrroloquinoxalines, imidazoles, oxathiines, thienotriazolopyrimidines, and thiazines exhibit anticancer activity [21]. Additionally, compounds that contain the 1,2,4-Triazole group have been recognized for their pharmacological activity as antitumor agents [70] and antiviral agents [71]. Likewise, anticancer activity was demonstrated for derivatives of 1,3,4-thiadiazole or 1,2,4-triazole linked to imidazoquinoxaline, spiroindoline, and thienopyrimidine [19,20].

(C) Therefore, the compounds **16**, **12a**, **12b**, **9**, and **5a-d** possess high effectiveness in treating specific carcinoma cell lines, particularly [MCF-7, breast adenocarcinoma cells] and [RPE-1, human normal retina-pigmented epithelium cells]. Besides, these compounds have various functional groups and moiety derivatives, as shown in (Fig. 4), which contributes to their superior cytotoxicity compared to the standard drug "Doxorubicin".

3. Molecular modeling

3.1. Computational material and methods

3.1.1. Simulation of molecular docking

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3.1.2. Synthesized protein receptors

The compounds' activity was inspected by downloading the Erα (Estrogen Receptor Alpha) (PDB: 5TZ1), Epidermal Growth Factor

5, a, R= Ar = C₆H₅
5, b, R= COCH₃,   Ar= *p*-C₆H₄-Cl
5, c, R= COCH₃,   Ar= *p*-C₆H₄-NO₂
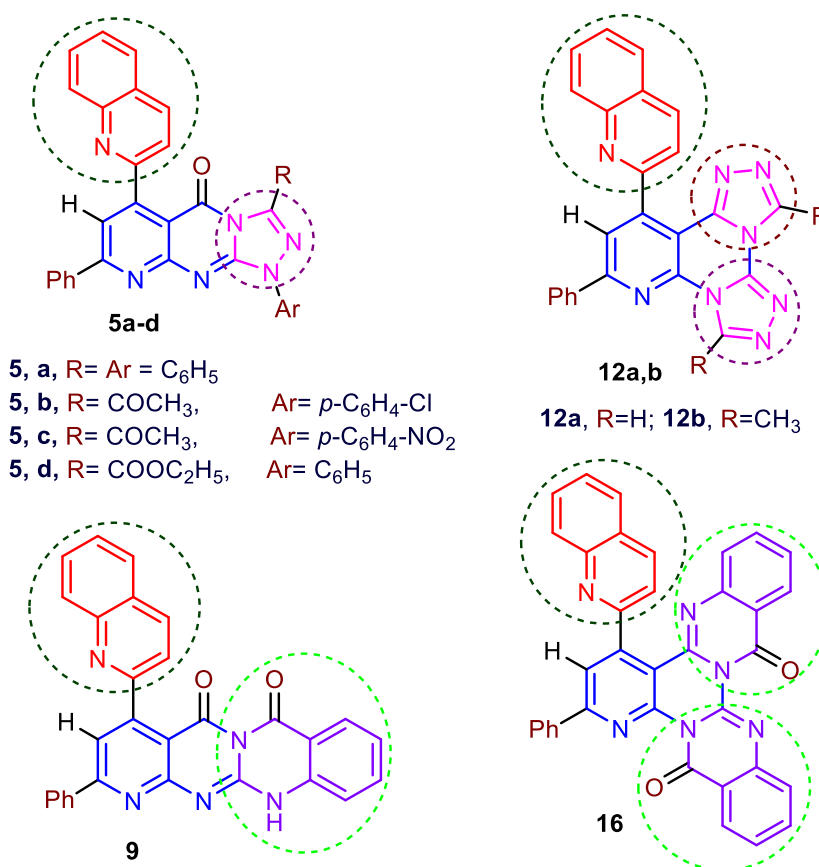**5, d,** R= COOC₂H₅,   Ar= C₆H₅

12a, R=H; **12b**, R=CH₃

Fig. 4. Some new compounds showed high cytotoxicity against carcinoma cell lines.

Receptor (PDB: 1M17), and NADPH oxidase protein (PDB: ID 6SZ5) from RCSB Protein Data Bank (Table 3). The crystal structures of the target were processed by removing water molecules, ions, and existing ligands using PyMOL software. The receptor molecule had hydrogen atoms added to it using MG Tools of Autodock Vina Eberhardt et al. [72]. This process was repeated for each protein and saved into a dockable pdbqt format for molecular docking. The complex inhibitors were separated from the crystal structures to be used as control ligands.

3.1.3. Synthesis of ligands

The Structure Data Format (SDF) of the compounds was obtained from the PubChem database. O'Boyle et al. [77] used Open Babel to convert each compound into a mol2 format. The Gasteiger method was employed to assign polar hydrogen charges, and the internal degrees of freedom and torsions were optimized to their minimum values. Autodock tools were used to convert the molecules to pdbqt format. Further, the chemical structures were subjected to MM2 energy minimization to enhance their stability.

3.2. Docking studies

Polar-H atoms were added to the target and Gasteiger charges were calculated using Au-todock tools prior to docking. The macromolecule file was saved in pdbqt format to prepare it for docking. Ligand-centered maps were generated by the AutoGrid program with grid dimensions of 90A° x 90A° x 90A°. All other parameters were set to default. Autodock Vina, developed by Eberhardt et al. [72], was utilized for docking experiments. The active site coordinates table (3) was used along with a grid box that was large enough to cover the entire protein structure to account for any possible protein-ligand interactions. The grid maps were calculated using Auto Grid to save a significant amount of time during docking [78,79].

3.2.1. Molecular interaction and visualization

Discovery Studio 4.5 was used to analyze the 2-D hydrogen-bond interactions in the target-ligand structure. The program provides graphical representations of hydrophobic and hydrogen bonds, including their lengths for each docking pose.

3.3. The results

3.3.1. Docking and molecular interaction studies with Erα (Estrogen Receptor Alpha)

Estrogen Receptor alpha (Erα) plays a critical role in breast tissue development, and bone metabolism. Docking results of compounds (9, 5b, 12a, 16 and 12b) showed the strongest affinity interaction with binding energies of −7.80, −8.70, −7.90, −8.60, −7.80 kcal/mol, respectively, compared with Doxorubicin as reference −6.40 kcal/mol. Only compound (9) formed hydrogen bonds with Pro324. Also, non-hydrophilic bonds were performed with compounds including (alkyl bonds) Val446, Pro406, Ile326, Pro325 and Trp393, (Pi-Anion) with Glu397 and Glu323, (C-Hydrogen bond) with Trp393 and Ile326, (Pi-cation) with Lys449, Glu323 and Arg 394, (Pi-Pi-T shaped) with Trp393, (Pi-Sigma) with Ile326, (Pi-Sulfur) with Met396. The residues Pro324, Glu353, and Leu387in the binding site were found to enhance the binding consanguinity. Total, the results suggest that compounds 9, 5b, 12a, 16, and 12b are the most promising candidates for further investigation as potential inhibitors of Estrogen Receptor alpha (Fig. 5 and Table 4).

3.3.2. Docking and molecular interaction studies with Butyrylcholinesterase

EGFR (Epidermal Growth Factor Receptor) is a receptor protein implicated in cell signaling and plays a significant role in cell growth, proliferation, and survival. Our docking analysis of compounds (9, 5b, 12a, 16 and 5c) showed the strongest affinity interaction with binding energies of −12.40, −11.50, −8.10, −12.20 and −11.20 kcal/mol, respectively, compared with Doxorubicin as reference −9.60 kcal/mol. Compounds formed hydrogen bond with Asp855 and Met793. Also, non-hydrophilic bonds were also observed with compounds including (alkyl bond) Leu788, Leu718, Leu844, Ala743, Lys745, rg841, Cys797 and Val726, (Pi-sigma) with Thr790, Val726, Leu718, Leu844, (Pi-Anion) with Asp855. The residues Asp855, Met793, and Leu844 in the catalytic site enhance binding affinity, suggesting potential for EGFR inhibitors (Fig. 6 and Table 5).

3.3.3. Docking and interaction studies with NADPH oxidase protein (PDB:ID 6SZ5)

NADPH oxidase is an enzyme complex that generates reactive oxygen species and has both beneficial and detrimental roles in cellular physiology and disease. After analyzing the docking results, compounds (16, 12a, 5c, 9 and 5b) have a strong affinity for NADPH oxidase, with a binding energy of −12.50, −11.00, −11.50, −12.70 and −10.30 kcal/mol, respectively. These compounds formed hydrogen bond with Ser178, His105, His194, Cys113, Lys574, Ser576, Asp99 and Arg102. Also, non-hydrophilic bonds were also observed including (alkyl bond) with Ile71, Met174, Ile112, Ile175, Leu80, Pro353, Arg102, His105, Met75, Ile106, Lys574,

Table 3

This is a list of target proteins, their corresponding PDB IDs, active site coordinates, native ligands, and references.

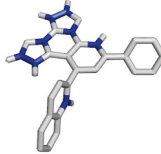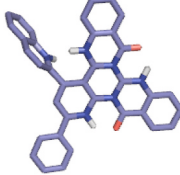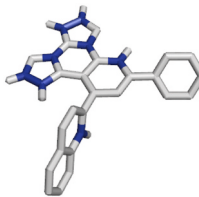| Protein Receptors		PDB ID	Resolution	Active site coordinates:			Reference Ligands	Reference
				X	Y	Z		
ERα	Erα (Estrogen Receptor Alpha)	**5TZ1**	1.90 Å	15.22	−4.14	25.33	**Doxorubicin**	[73]
EGFR(WT)	Epidermal Growth Factor Receptor	**1M17**	2.60 Å	4.36	33.47	−14.16	**Doxorubicin**	[74]
NADPH	NADPH oxidase protein (PDB:ID 6SZ5)	**6SZ5**	2.23 Å	5.24	−2.05	−3.09	**Vitamin C**	[75,76]
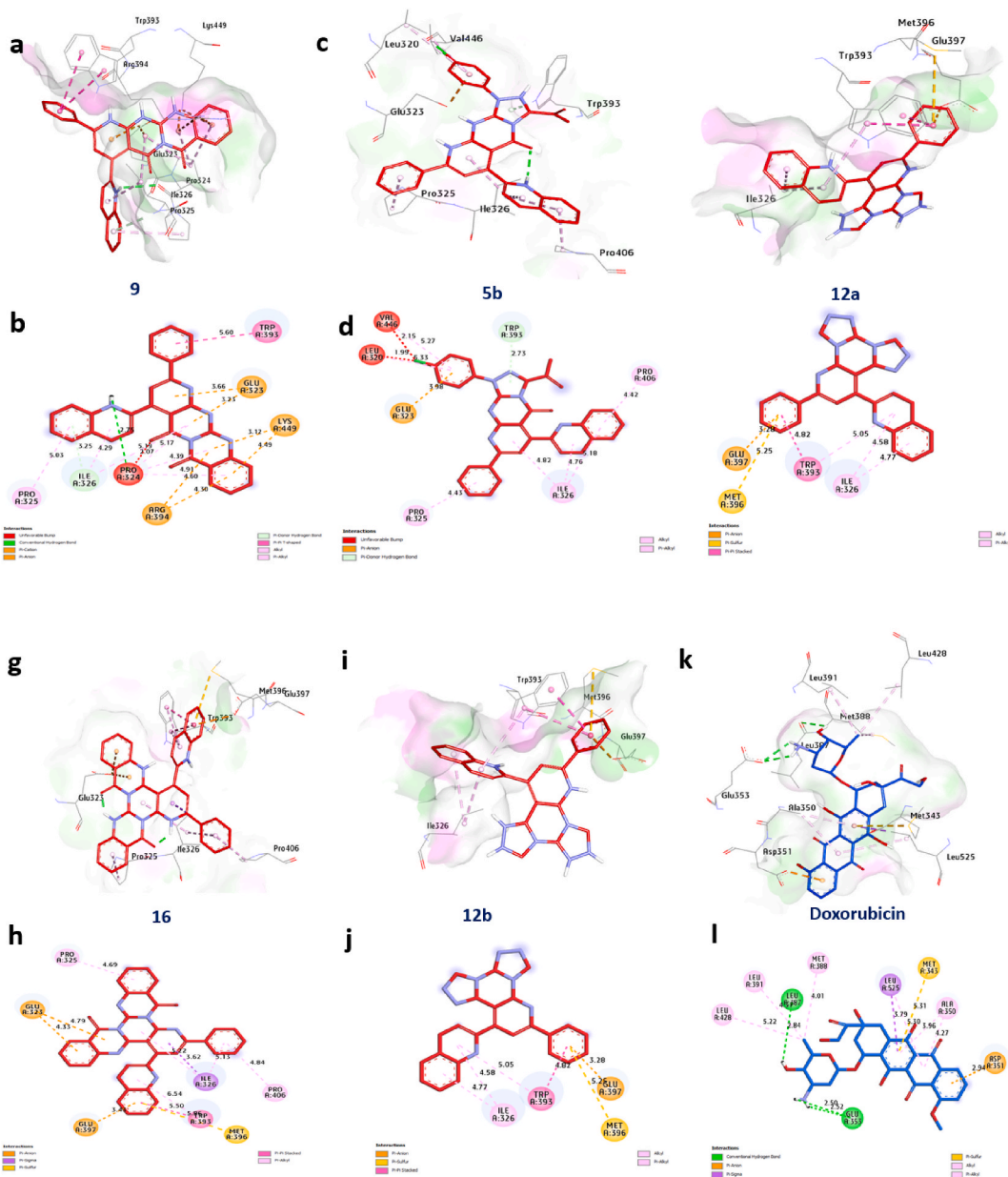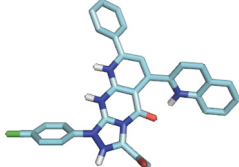
Fig. 5. 3D exemplifications of the compound's conformations are located at the binding pocket of the Erα (Estrogen Receptor Alpha) (PDB:ID 5TZ1) (a and b) 9, (c and d) 5b, (e and f) 12a, (g and h) 16, (I and j) 12b, (k and i) Doxorubicin.

Lys100, Ala81 and Arg77, (Pi-Pi T shaped) with Tyr338 and Phe200, (Pi-cation) with Arg102, Arg183, Arg77, Ile71 and His105, (Pi-sulfur) with Cys113, (Pi-Pi stacked) with Phe190 and Phe197, (carbon-H-Bond) with Asp99 and Tyr187, (Pi-sigma) with Phe197, Met75, Ile175, Ile71 and Met174. The residues Ser178, His105, His194, Asp99, and Arg102 in the catalytic site were found to enhance the binding affinity. Generally, the results indicate that compounds have potential as inhibitors of NADPH oxidase protein and warrant further investigation (Fig. 7 and Table 6).

4. Experimental section

The research plan involved a collaboration with a team to synthesize new heterocyclic compounds from pyrido [2,3-*d*] pyrimidine derivatives and evaluate their anti-cancer properties. The plan was executed successfully.

Table 4

The molecular interactions between ligands and amino acids of Erα (Estrogen Receptor Alpha) (PDB:ID 5TZ1).

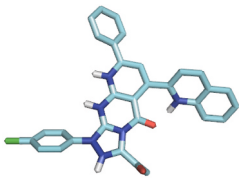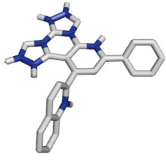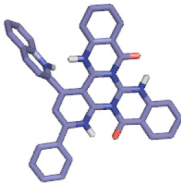| No	Protein	Ligand	3D Structure	Hydrophilic Interactions		Hydrophobic Contacts		No. of H-Bonds	No. of Total Bonds	affinity kcal mol-1
				Residue (H-Bond)	Length	Residue (Bond type)	Length			
1	Erα (Estrogen Receptor Alpha) (PDB:ID 5TZ1)	9		Pro324, (H-Bond)	2.75	Arg 394, (Pi-cation)	4.30	1	16	−7.80
						Lys449, (Pi-cation)	3.12			
						Glu323, (Pi-cation)	3.23			
						Trp393, (Pi-Pi-T shaped)	5.60			
							5.03			
						Pro325, (alkyl)	3.25			
						Ile326, (C-Hydrogen bond)				
2		5b		-	–	Val446, (Pi- alkyl)	5.27	0	10	−8.70
						Pro406, (Pi- alkyl)	4.42			
						Glu323, (Pi-Anion)	3.98			
						Ile326, (alkyl)	4.82			
						Pro325, (alkyl)	4.43			
						Trp393, (C-Hydrogen bond)	2.73			
3		12a		-	–	Glu323, (Pi-Anion)	3.28	0	6	−7.90
						Ile326, (alkyl)	4.77			
						Met396, (Pi-Sulfur)	5.25			
						Trp393, (Pi-Pi-T shaped)	4.82			
4		16		-	–	Pro325, (Pi-alkyl)	4.69	0	11	−8.60
						Pro406, (Pi-alkyl)	4.84			
						Glu323, (Pi-Anion)	4.33			
						Ile326, (alkyl)	5.13			
						Ile326, (Pi-Sigma)	5.22			
						Glu397, (Pi-Anion)	3.43			
						Met396, (Pi-Sulfur)	5.80			
						Trp393, (C-Hydrogen bond)	6.54			
5		12b		-	–	Trp393, (Pi-alkyl)	5.05	0	6	−7.80
						Ile326, (alkyl)	4.77			
						Ile326, (Pi- alkyl)	4.58			
						Glu397, (Pi-Anion)	3.28			
						Met396, (Pi-Sulfur)	5.25			
						Trp393, (C-Hydrogen bond)	4.82			
6		Doxorubicin		Glu353, (H-Bond)	2.50	Met 343, (Pi-sulfur)	5.31	3	12	−6.40
					2.52	Asp 351, (Pi-Anion)	2.94			
				Glu353, (H-Bond)	2.84	Leu 525, (Pi-sigma)	3.79			
				Leu 387, (H-Bond)		Leu 428, (Pi-alkyl)	5.22			
						Leu 391, (Pi-alkyl)	4.31			
						Met 388, (alkyl)	4.01			
						Ala 350, (Pi-alkyl)	3.96			
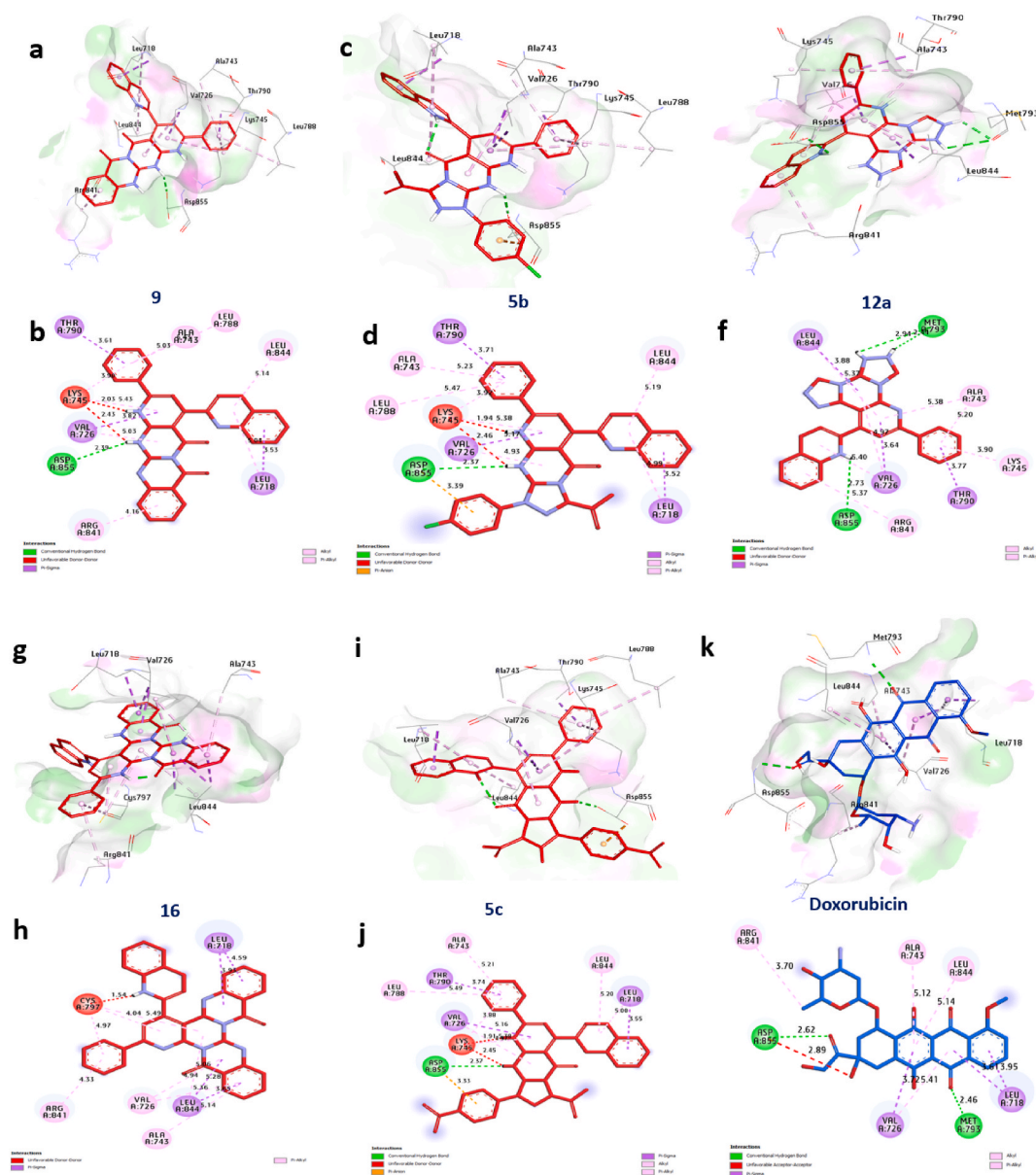
Fig. 6. 3D exemplifications of the compound's conformations are located at the binding pocket of EGFR (PDB:ID 4BDS). (a and b) 9, (c and d) 5b, (e and f) 12a, (g and h) 16, (I and j) 12b, (k and i) Doxorubicin.

4.1. General information

The Electrothermal IA 9100 series digital melting point apparatus, manufactured by Shimadzu in Tokyo, Japan, was used to measure the melting points of all substances. Elemental analyses were conducted using a Vario EL machine by Elementar in Langenselbold, Germany. Microanalytical data were processed by the microanalytical Centre of the Faculty of Science at Cairo University and the National Research Centre. The PerkinElmer 1650 spectrometer, located in Waltham, MA, USA, was used to record the IR spectra, which were measured using a KBr disc. JEOL 270 MHz and JEOL JMS-AX 500 MHz spectrometers made by JEOL in Tokyo, Japan were used to obtain NMR spectra, with Me$_4$Si serving as an internal standard. Mass spectra were recorded using an EI Ms-QP 1000 EX instrument made by Shimadzu in Tokyo, Japan, at 70 eV. The biological evaluations were carried out by the anticancer unit of Mansoura University's Faculty of Pharmacy (Department of Pharmacognosy) in 35516, Egypt. All starting materials and solvents were purchased from Sigma–Aldrich located in Saint Louis, MO, USA.
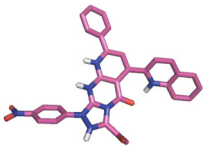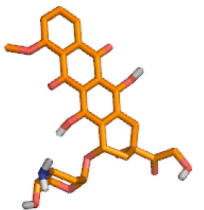
Table 5
Molecular interactions between ligands and amino acids of EGFR (PDB ID: 4BDS). Amino acids exhibiting similar interactions are highlighted in bold and red.

	Protein	Ligand	3D Structure	Hydrophilic Interactions		Hydrophobic Contacts		No. of H-Bonds	No. of Total Bonds	affinity kcal mol-1
				Residue (H- Bond)	Length	Residue (Bond type)	Length			
1	**EGFR (PDB: ID 4BDS)**	**9**		Asp855, (H-Bond)	2.39	Thr790, (Pi-sigma) Leu718, (Pi-sigma) Val726, (Pi-sigma) Leu844, (Pi-alkyl) Leu788, (Pi-alkyl) Ala743, ((Pi-alkyl) Arg 841, (Pi-alkyl) Leu718, (Pi-alkyl)	3.61 3.53 3.82 5.14 4.55 5.03 4.16 5.04	**1**	**14**	**−12.40**
2		**5b**		Asp855, (H-Bond)	2.37	Thr790, (Pi-sigma) Leu718, (Pi-sigma) Val726, (Pi-sigma) Leu844, (Pi-alkyl) Leu788, (Pi-alkyl) Ala743, ((Pi-alkyl) Val726, (Pi-alkyl) Asp855, (Pi-anion)	3.71 3.52 2.46 5.19 5.47 5.23 4.93 3.39	1	15	−11.50
3		**12a**		Asp855, (H-Bond) Met793, (H-Bond) Met793, (H-Bond)	2.73 2.94 2.88	Thr790, (Pi-sigma) Leu844, (Pi-sigma) Val726, (Pi-sigma) Lys745, (Pi-alkyl) Arg 841, (Pi-alkyl) Ala743, ((Pi-alkyl) Val726, (Pi-alkyl) Leu844, (Pi-alkyl)	3.77 3.88 3.64 3.90 5.37 4.97 5.37 4.322	**3**	**13**	**−8.10**
4		**16**		–	–	Leu718, (Pi-sigma) Leu844, (Pi-sigma) Val726, (Pi-alkyl) Cys797, (Pi-alkyl) Arg 841, (Pi-alkyl) Ala743, ((Pi-alkyl) Val726, (Pi-alkyl) Leu844, (Pi-alkyl)	4.59 3.65 4.94 4.04 4.33 5.14 4.94 5.06	**0**	**13**	**−12.20**

Table 5 (*continued*)

	Protein	Ligand	3D Structure	Hydrophilic Interactions		Hydrophobic Contacts		No. of H-Bonds	No. of Total Bonds	affinity kcal mol-1
				Residue (H- Bond)	Length	Residue (Bond type)	Length			
5	**5c**			Asp855, (H-Bond)	2.37	Thr790, (Pi-sigma)	3.74	**1**	**13**	**−11.20**
						Leu718, (Pi-sigma)	3.55			
						Val726, (Pi-sigma)	5.16			
						Leu844, (Pi-alkyl)	5.20			
						Leu788, (Pi-alkyl)	5.49			
						Ala743, ((Pi-alkyl)	5.21			
						Val726, (Pi-alkyl)	4.97			
						Asp855, (Pi-Anion)	3.33			
6	**Doxorubicin**			Asp855, (H-Bond)	2.62	Leu718, (Pi-sigma)	3.95	**2**	**10**	**−9.60**
				Met793, (H-Bond)	2.45	Val726, (Pi-sigma)	3.72			
						Leu844, (Pi-alkyl)	5.14			
						Arg 3.70, (Pi-alkyl)	3.70			
						Ala743, ((Pi-alkyl)	5.12			
						Val726, (Pi-alkyl)	5.41			

4.2. Synthesis of 7-phenyl-5-(quinolin-2-yl)-2-thioxo-2,3-dihydropyrido[2,3-d] pyrimidin-4(1H)-one (1), [54]

General Procedure [54]: A mixture of 1-phenyl-3-(quinoline) propenone (chalcone, 2.59g, 0.01 mol) and 6-amino-2-thioxopyrimidinone (1.43g, 0.01 mol) in 30 mL of dimethylformamide was heated under reflux for 18–20 h. Once the reaction was complete, confirmed by TLC, the solution was cooled to 0–5 °C, and the resulting solid was filtered out and recrystallized from DMF. The final product **1** was obtained as yellow crystals with an 85% yield.

4.3. Synthesis of 2-(methylthio)-7-phenyl-5-(quinolin-2-yl) pyrido [2,3-d] pyrimidin-4(3H)-one (2), [54]

Method A. General Procedure [54]: 3.82 g (0.01 mol) of Compound **1** were added to a warm solution of 0.01 mol KOH in 45 mL of ethanol. The mixture was heated for 40 min and then cooled down to room temperature. After that, 0.012 mol of methyl iodide was added to the solution. The solution was stirred under reflux for 6–8 h, then cooled down again and poured into 100 mL of cold water. The precipitated product was filtered, washed with water, and dried. Finally, the product was crystallized from DMF, resulting in a yield of 90% yellowish crystals.

Method B. To prepare compound **2**, dissolve 3.82 g (0.01 mol) of compound **1** in 35 mL of dimethylformamide. Then, add 1.40 g (0.01 mol) of anhydrous potassium carbonate and 1.40 mL (0.01 mol) of methyl iodide to the solution. Stir the resulting mixture at room temperature overnight. Dilute the solution with water and filter the solid formed, followed by washing it with water. Dry the product and then crystallize it from methanol. This process will result in the formation of yellowish crystals. The yield of the product was 80%.

4.4. Synthesis of substituted-phenyl-6-(quinolin-2-yl) pyrido [2,3-d] [1,2,4] triazolo [4,3-a] pyrimidin-5(1H)-one (5a-d)

The procedure for creating compounds **5a-d** generally involves the following steps. First, a solution of compound **1** (3.82g, 10 mmol) and the appropriate hydrazonoyl chlorides **3a-d** (10 mmol) should be stirred under reflux in dry chloroform (40 mL) and five drops of triethylamine for **7–9** h while monitoring with TLC. After that, the resulting solution should be evaporated under reduced pressure. The solid obtained should be washed several times with 40 mL of methanol and then crystallized from a suitable solvent to obtain **5a-d** in good yields.
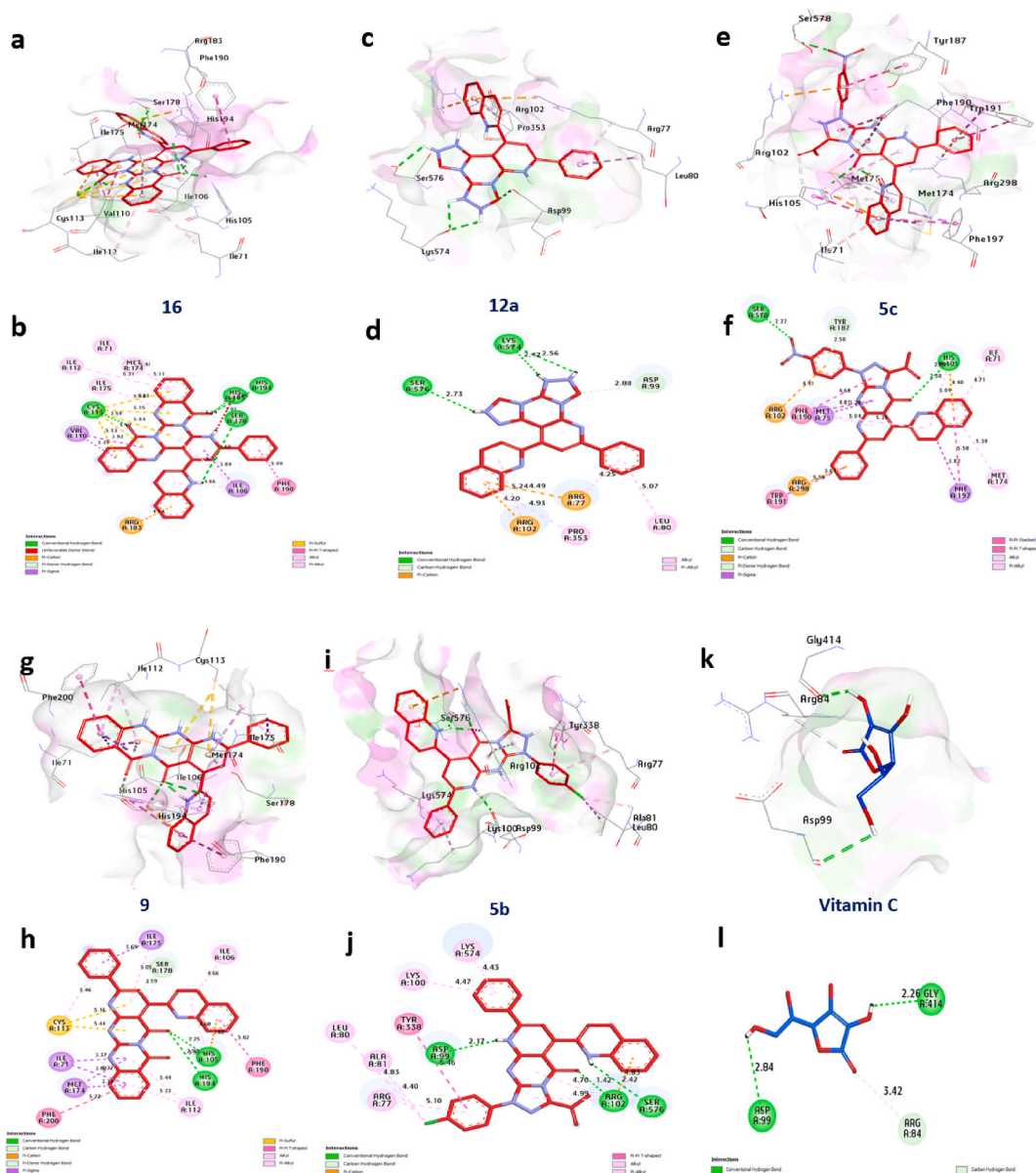
Fig. 7. 3D exemplifications of the compound conformations located at the binding pocket of the NADPH oxidase protein (PDB:ID 6SZ5), (a and b) 16, (c and d) 12a, (e and f) 5c, (g and h) 9, (I and j) 5b, (k and i) Vitamin C.
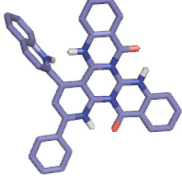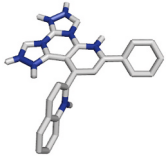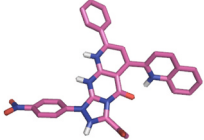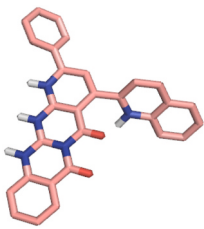
4.5. Synthesis of 1,3,8-triphenyl-6-(quinolin-2-yl) pyrido [2,3-d] [1,2,4] triazolo [4,3-a] pyrimidin-5(1H)-one (5a)

The yellowish crystals were obtained from **1** and *N*-phenylbenzene-carbohydrazonoyl chloride **3a** (2.31 g, 10 mmol). The compound was crystallized from DMF with an 80% yield and had a melting point of >350 °C (dec.); IR (ν, cm^{-1}) KBr: 3085 (CH aryl), 2955 (CH alkyl), 1685 (CO, amide), 1650 (C=N), 1580 (C=C); ^{1}H NMR (DMSO-d_6, ppm) δ 7.10–7.93 (m, 21H, three phenyl, quinoline rings), 8.05 (s, 1H, pyridine); ^{13}C NMR (DMSO-d_6) δ 105.8 (1C, CH, pyridine), 109.2, 110.5, 114.7116.5, 118.8, 120.4, 122.6, 126.5, 130.1, 131.7, 135.3, 137.5, 138.2, 139.4, 140.1, 140.7, 142.5, 146.8, 148.5, 151.2, 154.5, 155.6, 156.3, 157.9, 158.2, 161.1, 162.4 (33C, Ar–C),173.5 (1C, carbonyl group); MS (70 ev, %): *m/z* = 542 (M$^+$, 100%); Anal. Calc. for C$_{35}$H$_{22}$N$_6$O (542.60): C, 77.48 (77.41); H, 4.09 (4.15); N, 15.49 (15.55).

4.6. Synthesis of 3-acetyl-1-(4-chlorophenyl)-8-phenyl-6-(quinolin-2-yl) pyrido [2,3-d] [1,2,4] triazolo [4,3-a] pyrimidin-5(1H)-one (5b)
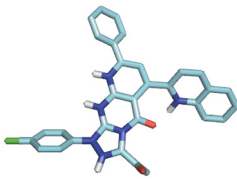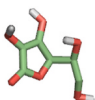
The yellow crystals of the compound were obtained from **1** and 2-oxo-*N*-(4-chloro-phenyl) propane hydrazonoyl chloride **3b** (1.96

Table 6
The molecular interactions of ligands with amino acids of NADPH oxidase protein.

No	Protein	Ligand	3D Structure	Hydrophilic Interactions		Hydrophobic Contacts		No. of H-Bonds	No. of Total Bonds	affinity kcal mol-1
				Residue (H- Bond)	Length	Residue (Bond type)	Length			
1	**NADPH oxidase protein**	**16**		Ser178, (H- Bond) His105, (H- Bond) His194, (H- Bond) Cys113, (H- Bond)	2.90 2.20 3.50 2.59	Ile106, (Pi-sigma) Val 110, (Pi-sigma) Ile71, (Pi-alkyl) Met174, (Pi-alkyl) Ile112, (Pi-alkyl) Ile175, (Pi-alkyl) Cys113, (Pi-sulfur) Phe190, (Pi-Pi stacked) Arg183, (Pi-cation)	3.89 3.29 5.41 5.11 5.31 2.35 5.15 5.00 4.14	**4**	**19**	**−12.50**
2		**12a**		Lys574, (H- Bond) Lys574, (H- Bond) Ser576, (H- Bond)	2.42 2.56 2.73	Leu80, (Pi-alkyl) Pro353, (Pi-alkyl) Arg102, (Pi-alkyl) Arg77, (Pi-alkyl) Arg102, (Pi-cation) Arg77, (Pi-cation) Asp99, (carbon-H-Bond)	5.07 4.93 5.22 4.25 4.49 4.20 2.88	**3**	**10**	**−11.00**
3		**5c**		Ser178, (H- Bond) His105, (H- Bond)	2.22 2.30	Phe197, (Pi-sigma) Met75, (Pi-sigma) Met174, (Pi-alkyl) Arg102, (Pi-alkyl) Ile71, (Pi-cation) His105, (Pi-alkyl) Met75, (Pi-alkyl) Phe197, (Pi-Pi stacked) Tyr187, (carbon-Bond)	3.82 3.78 5.38 4.41 4.71 5.09 5.04 5.58 2.50	**2**	**18**	**−11.50**
4		**9**		His105, (H- Bond) His194, (H- Bond)	2.25 2.94	Ile175, (Pi-sigma) Ile71, (Pi-sigma) Met174, (Pi-sigma) Ile106, (Pi-alkyl) His105, (Pi-alkyl) Ile175, (Pi-alkyl) Ile112, (Pi-	3.69 3.37 3.37 4.66 4.68 5.05 5.44 5.44 5.22 3.02	**2**	**20**	**−12.70**

(continued on next page)

Table 6 (*continued*)

No	Protein	Ligand	3D Structure	Hydrophilic Interactions		Hydrophobic Contacts		No. of H-Bonds	No. of Total Bonds	affinity kcal mol-1
				Residue (H- Bond)	Length	Residue (Bond type)	Length			
						alkyl) Cys113, (Pi-sulfur) Phe200, (Pi-Pi T shaped) His105, (Pi-cation)				
5		5b		Ser 578, (H- Bond) Asp99, (H-Bond) Arg102, (H- Bond)	2.42 2.17 1.42	Lys574, (Pi-alkyl) Lys100, (Pi-alkyl) Ala81, (Pi-alkyl) Arg77, (Pi-alkyl) Arg102, (Pi-alkyl) Leu80, (Pi-alkyl) Tyr338, (Pi-Pi T shaped) Arg102, (Pi-cation)	4.43 4.47 4.40 5.10 4.99 4.83 5.46 2.42	3	14	−10.30
6		Vitamin C		Asp99, (H-Bond) Gly 414, (H- Bond)	2.84 2.26	Arg 84, (carbon-H-Bond)	3.42	2	3	–9.70

g, 10 mmol). They were crystallized from dioxane with a yield of 78%. The melting point was greater than >350 °C and the crystals decomposed at that temperature; IR (ν, cm^{-1}) KBr: 3082 (CH aryl), 2953 (CH alkyl),1720,1680 (2C=O), 1645 (C=N), 1585(C=C); ^{1}H NMR (DMSO-d_6, ppm) δ 2.1 (s, 3H, CH$_3$),7.01–7.04 (d, 2H, J = 7.30 Hz, 4-chloro-phenyl),7.05–7.10 (d, 2H, J = 7.35 Hz, 4-chloro-phenyl), 7.15–7.91 (m, 11H, phenyl, quinoline ring), 8.30 (s, 1H, pyridine); ^{13}C NMR (DMSO-d_6) δ 22.7 (1C, CH$_3$),115.1 (1C, CH, pyridine),118.3, 119.1, 120.8121.5, 124.1, 125.7, 126.5, 126.9, 127.2, 128.1, 128.5, 129.4, 129.8, 135.3, 137.9, 142.2, 143.5, 149.7, 151.4, 153.2, 153.8, 155.5, 157.1 (27C, Ar–C),168.5, 175.7 (2C, two carbonyl group); MS (70 ev, %): m/z = 542 (M$^+$, 95%); Anal. Calc. for C$_{31}$H$_{19}$ClN$_6$O$_2$ (542.98): C, 68.57 (68.66); H, 3.53 (3.46); N, 15.48 (15.57).

4.7. Synthesis of 3-acetyl-1-(4-nitrophenyl)-8-phenyl-6-(quinolin-2-yl) pyrido [2,3-d] [1,2,4] triazolo [4,3-a] pyrimidin-5(1H)-one (5c)

The white crystals compound was obtained from **1** and 2-oxo-*N*-(4-nitrophenyl) propane hydrazonoyl chloride **3c** (2.06 g, 10 mmol). It was crystallized from methanol with a yield of 75% and had a melting point of >350 °C (dec.); IR (ν, cm^{-1}) KBr: 3082 (CH aryl), 2953 (CH alkyl),1720,1680 (2C=O), 1640 (C=N), 1590 (C=C); ^{1}H NMR (DMSO-d_6, ppm) δ 2.2 (s, 3H, CH$_3$),7.02–7.05 (d, 2H, J = 7.32 Hz, 4-nitro-phenyl),7.06–7.11 (d, 2H, J = 7.37 Hz, 4-nitrophenyl), 7.16–7.93 (m, 11H, phenyl, quinoline ring), 8.35 (s, 1H, pyridine); ^{13}C NMR (DMSO-d_6) δ 22.9 (1C, CH$_3$),116.2 (1C, CH, pyridine),118.5, 119.6, 120.2, 121.2, 124.8, 125.1, 126.3, 126.7, 127.4, 128.2, 129.5, 129.8, 136.5, 137.1, 138.6, 142.4, 144.1, 147.3, 151.8, 152.7, 153.9, 154.7, 157.3 (27C, Ar–C),169.1, 176.2 (2C, two carbonyl group); MS (70 ev, %): m/z = 553 (M$^+$, 90%); Anal. Calc. for C$_{31}$H$_{19}$N$_7$O$_4$ (553.54): C, 67.27 (67.35); H, 3.46 (3.40); N, 17.71 (17.80).

4.8. Synthesis of ethyl 5-oxo-1,8-diphenyl-6-(quinolin-2-yl)-1,5-dihydropyrido [2,3-d] [1,2,4] triazolo [4,3-a] pyrimidine-3-carboxylate (5d)

The yellowish crystals were obtained from **1** and chloro-(phenyl hydrazone) ethylacetate **3d** (2.27g, 10 mmol). The compound was crystallized from toluene with a yield of 70%. Its melting point was >350 °C (dec); IR (ν, cm^{-1}) KBr: 3078 (CH aryl), 2950 (CH alkyl),1750,1675 (2C=O), 1635 (C=N), 1575 (C=C); ^{1}H NMR (DMSO-d_6, ppm) δ 1.30 (t, 3H, J = 7.04 Hz, CH$_3$), 4.45 (q, 2H, J = 7.08 Hz, CH$_2$), 7.21–7.95 (m, 16H, two phenyl, quinoline ring), 8.40 (s, 1H, pyridine); ^{13}C NMR (DMSO-d_6) δ 18.5 (1C, CH$_3$), 68.2 (1C, CH$_2$),117.8 (1C, CH, pyridine),119.1, 119.8, 120.7, 122.1, 125.2, 126.5, 126.8, 127.1, 127.8, 128.1, 129.4, 129.7, 130.3, 137.4, 139.5, 140.8, 142.6, 144.9, 151.4, 152.3, 154.2, 154.6, 157.5 (27C, Ar–C),162.4, 168.7 (2C, two carbonyl group); MS (70 ev, %): m/z = 538 (M$^+$, 85%); Anal. Calc. for C$_{32}$H$_{22}$N$_6$O$_3$ (538.57): C, 71.37 (71.30); H, 4.12 (4.19); N, 15.60 (15.68).

4.9. Synthesis of 2-(methylsulfonyl)-7-phenyl-5-(quinolin-2-yl) pyrido [2,3-d] pyrimidin-4(3H)-one (6)

A solution containing **2** (3.96 g, 10 mmol) was mixed with an excess amount of hydrogen peroxide (6 mL) in glacial acetic acid (40 mL). The mixture was heated by stirring and refluxed for 9–12 h under controlled conditions (TLC). Once the reaction was complete, the mixture was cooled down to room temperature using ice water. The solid precipitate was filtered off and then crystallized from dimethylformamide to obtain yellow crystals with a yield of 72%. The melting point of the crystals was greater than >350 °C (dec); IR (ν, cm^{-1}) KBr: 3250 (br, NH), 3075 (CH aryl), 2940 (CH alkyl), 1677 (CO, amide), 1630 (C=N), 1582 (C=C), 1350, 1175, (SO$_2$); ^{1}H NMR (DMSO-d_6, ppm) δ 3.10 (s, 3H, SO$_2$CH$_3$),7.10–7.80 (m, 11H, phenyl, quinoline rings), 8.20 (s, 1H, pyridine), 11.70 (br., 1H, NH, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ 35.8 (1C, CH$_3$), 117.8 (1C, CH, pyridine), 119.4, 120.5, 121.3, 125.1, 126.5, 126.8, 127.5, 128.1, 129.3, 129.8, 136.7, 139.2, 140.2, 145.1, 150.9, 154.3, 155.4, 156.7 (20C, Ar–C), 162.5 (1C, carbonyl group); MS (70 ev, %): m/z = 428 (M$^+$, 100%); Anal. Calc. for C$_{23}$H$_{16}$N$_4$O$_3$S (428.47): C, 64.47 (64.55); H, 3.76 (3.70); N, 13.08 (13.15).

4.10. Synthesis of 2-substituted-7-phenyl-5-(quinolin-2-yl) pyrido [2,3-d] pyrimidin-4(3H)-one (7a, b)

General Procedure: To prepare compound **2** (3.96g, 10 mmol), it should be dissolved in 80 mL of absolute methanol and then added to a freshly distilled sec-amine (10 mmol), like piperazine or morpholine. The resulting mixture should be stirred under reflux for 4–7 h and monitored using TLC. Afterward, let it cool to 0 °C for 12 h. The product should then be filtered, washed with 100 mL of water, dried, and recrystallized from the appropriate solvent to obtain the compound **(7a, b)**.

4.11. Synthesis of 7-phenyl-2-(piperazin-1-yl)-5-(quinolin-2-yl) pyrido [2,3-d] pyrimidin-4(3H)-one (7a)

The compound was synthesized from **2** and piperazine (0.86 g, 10 mmol) to yield yellowish crystals. The crystals were then recrystallized from dioxane with an 82% yield. The melting point was greater than >350 °C (dec.); IR (ν, cm^{-1}) KBr: 3370-3255 (br, 2NH), 3070 (CH aryl), 2940 (CH alkyl), 1681 (CO, amide), 1627 (C=N), 1577 (C=C); ^{1}H NMR (DMSO-d_6, ppm) δ 2.77–2.90 (t, 4H, 2CH$_2$, J = 7.02 Hz, piperazine), 3.75–3.90 (t, 4H, 2CH$_2$, J = 7.03 Hz, piperazine),7.10–8.10 (m, 11H, phenyl, quinoline rings), 8.30 (s, 1H, pyridine), 11.50 (br., 1H, NH, D$_2$O exchangeable), 11.70 (br., 1H, NH, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ 27.5, 29.9 (4C, 4CH$_2$, piperazine), 106.6 (1C, CH, pyridine), 115.7, 119.5, 120.1, 121.3, 124.7, 127.1, 128.5, 129.9, 130.5, 131.4, 132.6, 138.1139.5, 142.7, 148.3, 151.5, 152.8, 155.5 (20C, Ar–C), 165.7 (1C, carbonyl group); MS (70 ev, %): m/z = 434 (M$^+$, 90%); Anal. Calc. for C$_{26}$H$_{22}$N$_6$O (434.50): C, 71.87 (71.80); H, 5.10 (5.17); N, 19.34 (19.40).

4.12. Synthesis of 2-morpholino-7-phenyl-5-(quinolin-2-yl) pyrido [2,3-d] pyrimidin-4(3H)-one (7b)

The compound was synthesized by reacting **2** with morpholine (0.87g, 10 mmol) to obtain pale-yellow crystals. The product was then crystallized from ethanol to yield 73%. The melting point was greater than >350 °C and decomposition occurred; IR (ν, cm^{-1}) KBr: 3360 (br, NH), 3074 (CH aryl), 2944 (CH alkyl), 1687 (CO, amide), 1629 (C=N), 1578 (C=C); ^{1}H NMR (DMSO-d_6, ppm) δ 3.05–3.13 (t, 4H, 2CH$_2$, J = 7.01 Hz, morpholine), 3.60–3.68 (t, 4H, 2CH$_2$, J = 7.04 Hz, morpholine),7.22–7.92 (m, 11H, phenyl, quinoline rings), 7.96 (s, 1H, pyridine),10.45 (br., 1H, NH, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ 37.6, 38.4 (4C, 4CH$_2$, morpholine), 118.5 (1C, CH, pyridine), 119.4, 120.1, 121.3, 125.6, 126.9, 127.2, 127.9, 128.2, 129.4, 129.8, 137.1, 139.3145.1, 151.5, 152.3, 154.1, 155.2, 156.5 (20C, Ar–C), 162.3 (1C, carbonyl group); MS (70 ev, %): m/z = 435 (M$^+$, 88%); Anal. Calc. for C$_{26}$H$_{21}$N$_5$O$_2$(435.49): C, 71.71 (71.63); H, 4.86 (4.92); N, 16.08 (16.15).

4.13. Synthesis of 2-((4-oxo-7-phenyl-5-(quinolin-2-yl)-3,4-dihydropyrido[2,3-d] pyrimidin-2-yl) amino) benzoic acid (8)

A mixture of compound **2** (3.96g, 10 mmol) was added to 50 mL of absolute methanol and then combined with a freshly distilled primary amine called anthranilic acid (1.37g, 10 mmol). The solution was stirred and heated under reflux for 5–8 h while being monitored with thin-layer chromatography (TLC). After that, it was cooled on ice and left to sit for 12 h. The resulting product was then filtered, washed with water, and dried. It was recrystallized from dimethylformamide, resulting in yellow crystals with an 85% yield and melting point greater than >350 °C (decomposes); IR (ν, cm^{-1}) KBr: 3510 (br, OH), 3380, 3350 (br, 2NH), 3065 (CH aryl), 2941 (CH alkyl),1722, 1682 (2C=O), 1621 (C=N), 1575 (C=C); ^{1}H NMR (DMSO-d_6, ppm) δ 7.10–8.30 (m, 15H, two phenyl, quinoline rings), 8.50 (s, 1H, pyridine), 11.06 (br., 1H, NH, D$_2$O exchangeable),11.50 (br., 1H, NH, D$_2$O exchangeable), 11.70 (br., 1H, OH, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ 117.5 (1C, CH, pyridine), 117.8, 118.1, 118.5, 118.8, 119.4, 121.1, 125.7, 127.1, 127.4, 127.8, 128.1, 129.5, 129.7, 130.7, 135.1, 137.3, 139.5, 145.2, 149.6, 151.1, 151.9, 154.4, 155.3, 156.7 (26C, Ar–C), 162.8, 170.2 (2C, two carbonyl group); MS (70 ev, %): m/z = 485 (M$^+$, 100%); Anal. Calc. for C$_{29}$H$_{19}$N$_5$O$_3$(485.50): C, 71.74 (71.80); H, 3.94 (3.88); N, 14.43 (14.50).

4.14. Synthesis of 2-phenyl-4-(quinolin-2-yl)-5H-pyrido [2′,3′:4,5] pyrimido [2,1-b] quinazoline-5,7(12H)-dione (9)

A mixture consisting of compound **8** (4.85, 10 mmol) was prepared in 50 mL of glacial acetic acid. A small amount of sulfuric acid (1 mL) was added to the mixture, which was then heated and stirred under reflux for 9–12 h while being monitored using thin-layer chromatography (TLC). The reaction solution was allowed to cool and then poured into a mixture of crushed ice and water, then neutralized with an ammonia solution. The final product precipitate was filtered off, washed with water, and dried. The yellowish

crystals obtained were then purified by crystallization from dioxane, resulting in a yield of 73% of the final product, which had a melting point greater than >350 °C (decomposes); IR (ν, cm^{-1}) KBr: 3360 (br, NH), 3052 (CH aryl), 2947 (CH alkyl),1690, 1684 (2C=O), 1628 (C=N), 1583 (C=C); ^{1}H NMR (DMSO-d_6, ppm) δ 7.04–8.10 (m, 15H, two phenyl, quinoline rings), 8.65 (s, 1H, pyridine), 11.70 (br., 1H, NH, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ 117.2 (1C, CH, pyridine), 117.6, 118.2, 118.6, 118.9, 119.7, 121.4, 125.8, 126.9, 127.5, 127.9, 128.2, 128.4, 129.3, 129.6, 133.1137.2, 139.4, 145.1, 147.3, 150.9, 153.5, 154.6, 155.1, 156.4 (26C, Ar–C), 167.5, 167.8 (2C, two carbonyl group); MS (70 ev, %): m/z = 467 (M$^+$, 95%); Anal. Calc. for C$_{29}$H$_{17}$N$_5$O$_2$ (467.49): C, 74.51 (74.60); H, 3.67 (3.60); N, 14.98 (14.92).

4.15. Synthesis of 4-chloro-2-(methylthio)-7-phenyl-5-(quinolin-2-yl) pyrido [2,3-d] pyr imidine (10)

We combined **2** (3.96g, 10 mmol) with 40 mL of dry dioxane and added 12 mL of phosphorus oxychloride. The mixture was then heated and stirred under reflux for 5–8 h while using TLC to monitor the reaction progress. After cooling the mixture to room temperature, we poured it into cold water. This caused a precipitate to form, which was filtered and crystallized in methanol. Yellow crystals resulted. The yield of the crystals obtained was 85%, m. p. > 350 °C (dec.); IR (ν, cm^{-1}) KBr: 3062 (CH, aryl), 2938 (CH alkyl), 1618 (C=N), 1576 (C=C), 1170 (C–Cl); ^{1}H NMR (DMSO-d_6, ppm) δ 1.25 (s, 3H, SCH$_3$),7.10–8.10 (m, 11H, phenyl, quinoline rings), 8.20 (s, 1H, pyridine);^{13}C NMR (DMSO-d_6) δ 15.1 (1C, CH$_3$), 118.2 (1C, CH, pyridine), 118.7, 119.4, 121.7, 125.1, 127.1, 127.4, 127.9, 128.4129.6, 129.8, 137.3, 139.2, 144.9, 150.4, 154.5, 154.9, 155.3, 159.2, 161.9 (21C, Ar–C); MS (70 ev, %): m/z = 414 (M$^+$, 100%); Anal. Calc. for C$_{23}$H$_{15}$ClN$_4$S (414.91): C, 66.58 (66.51); H, 3.64 (3.70); N, 13.50 (13.42).

4.16. Synthesis of 2,4-dihydrazinyl-7-phenyl-5-(quinolin-2-yl) pyrido [2,3-d] pyrimidine (11)

A solution of **10** (4.14g, 10 mmol) and hydrazine hydrate (20 mL) was heated and stirred in a solution of 55 mL of dioxane and 25 mL of ethanol for a period of 11–15 h. The reaction was monitored using thin-layer chromatography (TLC). After completion of the reaction, the solution was cooled to 0 °C for 5–8 h and the resulting precipitate was filtered. The crystals obtained were yellowish and were crystallized from dioxane, resulting in a yield of 80%. The melting point of the crystals was greater than >350 °C (dec.); IR (ν, cm^{-1}) KBr: 3440–3345(brs, 2NH–NH$_2$), 3055 (CH, aryl), 2935 (CH alkyl), 1621 (C=N), 1574 (C=C); ^{1}H NMR (DMSO-d_6, ppm) δ 6.70 (br, NH$_2$, D$_2$O exchangeable), 6.80 (br, NH$_2$, D$_2$O exchangeable),7.01–8.10 (m, 11H, phenyl, quinoline rings), 8.35 (s, 1H, pyridine), 10.90, 11.80 (two, brs, 2NH, D$_2$O exchangeable);^{13}C NMR (DMSO-d_6) δ 116.9 (1C, CH, pyridine), 117.2, 119.5, 121.1, 125.6, 127.2, 127.5, 127.8, 128.2129.4, 129.7, 137.5, 139.3, 144.8, 153.9, 154.7, 155.3, 155.5, 158.7, 166.5 (21C, Ar–C); MS (70 ev, %): m/z = 394 (M$^+$, 100%); Anal. Calc. for C$_{22}$H$_{18}$N$_8$ (394.44): C, 66.99 (66.90); H, 4.60 (4.68); N, 28.41 (28.34).

4.17. Synthesis of substituted-10-phenyl-12-(quinolin-2-yl) pyrido [3,2-e] bis ([1,2,4] triazolo) [4,3-a:4',3'-c] pyrimidine (12a, b)

General Procedure: Mix 3.94 g (10 mmol) of compound **11** with 50 mL of (formic acid or glacial acetic acid). Heat the mixture beneath reflux for 17–22 h while monitoring with TLC. Allow the reaction mixture to cool to room temperature. After that, pour it into cold water and collect the precipitate by filtration. Wash the precipitate with 30 mL of ethanol and dry it. Finally, recrystallize the product (**12a, b**) from a suitable solvent.

4.18. Synthesis of 10-phenyl-12-(quinolin-2-yl) pyrido [3,2-e] bis ([1,2,4] triazolo) [4,3-a:4',3'-c] pyrimidine (12a)

The compound was synthesized from **11** and formic acid, resulting in white crystals which were recrystallized from methanol with a yield of 79%. The melting point is above >350 °C (dec.); IR (ν, cm^{-1}) KBr: 3052 (CH aryl), 2933 (CH alkyl), 1618 (C=N), 1570 (C=C); ^{1}H NMR (DMSO-d_6, ppm) δ 7.07–8.10 (m, 11H, phenyl, quinoline rings), 8.37 (s, 1H, pyridine), 8.50, 8.75 (2s, 2H, two triazole rings); ^{13}C NMR (DMSO-d_6) δ 116.5 (1C, CH, pyridine), 118.7, 120.1, 120.9, 125.1, 126.8, 127.4, 127.5, 128.2129.1, 129.6, 136.2, 137.7, 139.4, 144.5, 148.3, 152.1, 154.7, 154.9, 155.4, 157.5 (23C, Ar–C); MS (70 ev, %): m/z = 414 (M$^+$, 98%); Anal. Calc. for C$_{24}$H$_{14}$N$_8$ (414.43): C, 69.56 (69.50); H, 3.41 (3.48); N, 27.04 (27.12).

4.19. Synthesis of 3,7-dimethyl-10-phenyl-12-(quinolin-2-yl) pyrido [3,2-e] bis ([1,2,4] triazolo) [4,3-a:4',3'-c] pyrimidine (12b)

The compound was obtained as yellow crystals from **11** and glacial acetic acid, and then crystallized from dioxane (77% yield), m. p. > 350 °C (dec.); IR (ν, cm^{-1}) KBr: 3060 (CH aryl), 2941 (CH alkyl), 1624 (C=N), 1577 (C=C); ^{1}H NMR (DMSO-d_6, ppm) δ 2.15 (s, 6H, 2CH$_3$), 7.14–7.93 (m, 11H, phenyl, quinoline rings), 8.05 (s, 1H, pyridine);^{13}C NMR (DMSO-d_6) δ 17.9 (2C, 2CH$_3$), 117.7 (1C, CH, pyridine),119.6, 120.2, 121.4, 125.8, 126.9, 127.2, 127.7, 128.1, 129.4, 129.8, 136.9139.1, 144.3, 147.1, 149.8, 152.3, 154.5, 154.7, 155.2, 157.4 (23C, Ar–C); MS (70 ev, %): m/z = 442 (M$^+$, 92%); Anal. Calc. for C$_{26}$H$_{18}$N$_8$ (442.49): C, 70.58 (70.50); H, 4.10 (4.17); N, 25.32 (25.40).

4.20. Synthesis of N^2, N^4-bis(substituted)-7-phenyl-5-(quinolin-2-yl) pyrido [2,3-d] pyrimidine-2,4-diamine (13a-c)

General Method: In 50 mL of glacial acetic acid, dissolve 4.14g (10 mmol) of 10, then add 20 mmol of freshly distilled arylamine, such as aniline, 4-chloroaniline or 4-methoxyaniline. Stir and reflux the mixture for 10–13 h while monitoring its progress through TLC. Then, let the mixture cool to zero °C for 5–6 h. Filter the precipitate, wash it with water, and dry it. Recrystallize from a suitable

solvent to obtain **13a-c**.

4.21. Synthesis of N², N⁴,7-triphenyl-5-(quinolin-2-yl) pyrido [2,3-d] pyrimidine-2,4-diamine (*13a*)

The compound was synthesized by reacting aniline (1.86g, 20 mmol) with compound **10**, and subsequently crystallized from DMF to yield brownish crystals (71% yield). The melting point was observed to be above >350 °C, after which the compound decomposed; IR (ν, cm^{-1}) KBr: 3370, 3352 (br s, 2NH), 3058 (CH, aryl), 2931 (CH alkyl), 1615 (C=N), 1582 (C=C); ^{1}H NMR (DMSO-d_6, ppm) δ 6.90–7.90 (m, 21H, three phenyl, quinoline rings), 8.08 (s, 1H, pyridine), 10.40 (br, NH, D$_2$O exchangeable); 10.80 (br, NH, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ 113.3 (1C, CH, pyridine), 117.8, 121.5, 121.7, 121.9, 125.7, 128.1, 128.5, 129.3, 130.5, 133.8, 135.5, 136.2137.4, 140.1, 142.3, 144.5, 147.6, 149.4, 151.7, 154.8, 157.5, 158.7, 160.1, 160.2, 163.4, 166.2, 167.7 (33C, Ar–C); MS (70 ev, %): m/z = 516 (M$^+$, 95%); Anal. Calc. for C$_{34}$H$_{24}$N$_6$ (516.61): C, 79.05 (79.12); H, 4.68 (4.60); N, 16.27 (16.35).

4.22. Synthesis of N², N⁴-bis(4-chlorophenyl)-7-phenyl-5-(quinolin-2-yl) pyrido [2,3-d] pyrimidine-2,4-diamine (*13b*)

This compound was obtained from **10** and 4-chloroaniline (2.54g, 20 mmol), crystallized from dioxane (75% yield), yellowish crystals and m. p. > 350 °C (dec.); IR (ν, cm^{-1}) KBr: 3375, 3361 (br s, 2NH), 3053 (CH, aryl), 2937 (CH alkyl), 1622 (C=N), 1586 (C=C), 1180 (C–Cl); ^{1}H NMR (DMSO-d_6, ppm) δ 7.01–7.05 (d, 2H, J = 7.10 Hz, 4-chlorophenyl), 7.07–7.12 (d, 2H, J = 7.13 Hz, 4-chlorophenyl), 7.14–7.19 (d, 2H, J = 7.12 Hz, 4-chlorophenyl)), 7.20–7.25 (d, 2H, J = 7.14 Hz, 4-chlorophenyl), 7.27–7.98 (m, 11H, phenyl, quinoline rings), 8.07 (s, 1H, pyridine), 8.40 (br, NH, D$_2$O exchangeable); 9.65 (br, NH, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ 115.5 (1C, CH, pyridine), 116.6, 118.7, 120.6, 121.5, 121.8, 125.3, 126.8, 127.2, 127.7, 127.8, 127.9, 128.1128.7, 129.5, 129.7, 130.2, 137.1, 137.5, 139.2, 139.4, 144.6, 154.5, 154.7, 155.2, 155.4, 157.1, 166.5 (33C, Ar–C); MS (70 ev, %): m/z = 585 (M$^+$, 92%); Anal. Calc. for C$_{34}$H$_{22}$Cl$_2$N$_6$ (585.49): C, 69.75 (69.82); H, 3.79 (3.70); N, 14.35 (14.28).

4.23. Synthesis of N², N⁴-bis(4-methoxyphenyl)-7-phenyl-5-(quinolin-2-yl) pyrido [2,3-d] pyrimidine-2,4-diamine (*13c*)

This compound was obtained from **10** and 4-methoxyaniline (2.46g, 20 mmol), crystallized from THF (74% yield), yellow crystals and m. p. > 350 °C (dec.); IR (ν, cm^{-1}) KBr: 3372, 3357 (br s, 2NH), 3048 (CH, aryl), 2931 (CH alkyl), 1619 (C=N), 1583 (C=C); ^{1}H NMR (DMSO-d_6, ppm) δ 3.77 (s, 3H, CH$_3$), 3.85 (s, 3H, CH$_3$), 7.02–7.06 (d, 2H, J = 7.04 Hz, 4-methoxyphenyl), 7.08–7.13 (d, 2H, J = 7.05 Hz, 4-methoxyphenyl), 7.15–7.20 (d, 2H, J = 7.07 Hz, 4-methoxyphenyl), 7.21–7.26 (d, 2H, J = 7.09 Hz, 4-methoxyphenyl), 7.28–7.99 (m, 11H, phenyl, quinoline rings), 8.09 (s, 1H, pyridine), 8.51 (br, NH, D$_2$O exchangeable); 9.72 (br, NH, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ 51.80 (1C, OCH$_3$), 52.10 (1C, OCH$_3$),115.3 (1C, CH, pyridine), 116.1, 116.5, 116.8, 118.9, 119.5, 120.8, 121.2, 125.1, 126.9, 127.1, 127.4, 128.2129.3, 129.7, 130.9, 132.5, 137.4, 139.1, 144.5, 153.1, 153.5, 154.3, 154.7, 155.4, 155.6, 157.5, 167.1 (33C, Ar–C); MS (70 ev, %): m/z = 576 (M$^+$, 91%); Anal. Calc. for C$_{36}$H$_{28}$N$_6$O$_2$ (576.66): C, 74.98 (74.91); H, 4.89 (4.95); N, 14.57 (14.50).

4.24. Synthesis of 7-phenyl-2,4-di (piperazin-1-yl)-5-(quinolin-2-yl) pyrido [2,3-d] pyrimidine (*14a*) and 4,4'-(7-phenyl-5-(quinolin-2-yl) pyrido [2,3-d] pyrimidine-2,4-diyl) dimorpholine (*14b*)

General Procedure: A compound **10** (4.14 g, 10 mmol) was mixed with glacial acetic acid (55 mL) and then freshly distilled sec-amine (20 mmol), such as piperazine or morpholine, was added. The reaction solution was stirred and refluxed for 9–12 h while monitoring its progress with thin layer chromatography (TLC). After the reaction was complete, the solution was allowed to cool to 0 °C for 5 h, and the final precipitate was filtered, washed with water/ethanol, dried, and recrystallized from the appropriate solvent to produce **14a, b**.

4.25. Synthesis of 7-phenyl-2,4-di (piperazin-1-yl)-5-(quinolin-2-yl) pyrido [2,3-d] pyrimidine (*14a*)

This compound was obtained from **10** and piperazine (1.72g, 20 mmol), crystallized from methanol (76% yield), yellowish crystals and m. p. > 350 °C (dec.); IR (ν, cm^{-1}) KBr: 3379, 3366 (br s, 2NH), 3060 (CH, aryl), 2942 (CH alkyl), 1625 (C=N), 1590 (C=C); ^{1}H NMR (DMSO-d_6, ppm) δ 1.70–1.80 (t, 4H, J = 6.02 Hz, 2CH$_2$, piperazine), 2.10–2.20 (t, 4H, J = 6.03 Hz, 2CH$_2$, piperazine), 2.70–2.80 (t, 4H, J = 6.05 Hz, 2CH$_2$, piperazine), 3.75–3.85 (t, 4H, J = 6.06 Hz, 2CH$_2$, piperazine),7.10–8.09 (m, 11H, phenyl, quinoline rings), 8.25 (s, 1H, pyridine), 11.85 (br, NH, D$_2$O exchangeable), 12.02 (br, NH, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ 37.2, 37.5, 38.4, 38.9 (8C, piperazine),116.4 (1C, CH, pyridine), 117.8, 118.9, 120.7, 125.4, 127.1, 127.4, 127.7, 128.2, 129.5, 129.8, 137.3, 139.5, 144.2, 154.1, 154.6, 155.5157.5, 162.4168.1 (21C, Ar–C); MS (70 ev, %): m/z = 502 (M$^+$, 85%); Anal. Calc. for C$_{30}$H$_{30}$N$_8$ (502.63): C, 71.69 (71.60); H, 6.02 (6.10); N, 22.29 (22.23).

4.26. Synthesis of 4,4'-(7-phenyl-5-(quinolin-2-yl) pyrido [2,3-d] pyrimidine-2,4-diyl) dimorpholine (*14b*)

This compound was obtained from **10** and morpholine (1.74g, 20 mmol), crystallized from dioxane (69% yield), yellow crystals and m. p. > 350 °C (dec.); IR (ν, cm^{-1}) KBr: 3062 (CH, aryl), 2944 (CH alkyl), 1627 (C=N), 1593 (C=C); ^{1}H NMR (DMSO-d_6, ppm) δ 1.90–1.97 (t, 4H, J = 6.10 Hz, 2CH$_2$, morpholine), 1.99–2.05 (t, 4H, J = 6.12 Hz, 2CH$_2$, morpholine), 2.98–3.05 (t, 4H, J = 6.15 Hz, 2CH$_2$, morpholine), 3.08–3.14 (t, 4H, J = 6.18Hz, 2CH$_2$, morpholine), 7.04–7.97 (m, 11H, phenyl, quinoline rings), 8.03 (s, 1H,

pyridine); ^{13}C NMR (DMSO-d_6) δ 39.5, 39.9, 50.2, 50.8 (8C, morpholine),116.7 (1C, CH, pyridine), 118.3, 119.1, 121.2, 125.6, 126.9, 127.2, 127.8, 128.1, 129.4, 129.7, 137.5, 139.1, 144.4, 154.3, 154.7, 155.2, 157.4, 162.1168.5 (21C, Ar–C); MS (70 ev, %): m/z = 504 (M$^+$, 84%); Anal. Calc. for $C_{30}H_{28}N_6O_2$ (504.59): C, 71.41 (71.48); H, 5.59 (5.52); N, 16.66 (16.72).

4.27. Synthesis of 2,2'-((7-phenyl-5-(quinolin-2-yl) pyrido [2,3-d] pyrimidine-2,4-diyl) bis(azanediyl)) dibenzoic acid (15)

A mixture of compound **10** (4.14g, 10 mmol) in 70 mL of methanol or 60 mL of glacial acetic acid was added to freshly prepared anthranilic acid (2.74 g, 20 mmol). The solution was refluxed by stirring for 8–11 h, while monitoring the reaction progress with thin layer chromatography (TLC). After the reaction was completed, the solution was cooled down to zero °C and left to sit for 10–12 h. The solid that formed was filtered out, washed with water, and dried. The resulting solid was then recrystallized from DMF, which produced pale yellow crystals with an 82% yield. The melting point was greater than >350 °C (decomposition); IR (ν, cm^{-1}) KBr: 3500-3480 (br, two OH), 3380-3350 (br, two NH), 3090 (CH, aryl), 2950 (CH alkyl), 1720, 1715 (two C=O), 1614 (C=N), 1584 (C=C); ^{1}H NMR (DMSO-d_6, ppm) δ 7.12–8.30 (m, 19H, three phenyl, quinoline rings), 8.50 (s, 1H, pyridine), 11.50 (br, NH, D$_2$O exchangeable), 11.60 (br, NH, D$_2$O exchangeable),12.10 (br s, OH, D$_2$O exchangeable), 12.85 (br s, OH, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ 115.3 (1C, CH, pyridine), 115.7, 116.1, 116.4, 116.8, 117.2, 117.9, 118.7, 119.5, 120.8, 125.2, 126.8, 127.2, 127.7, 128.1129.4, 129.8, 131.2, 131.4, 134.3, 134.5, 137.4, 139.1142.8, 144.5, 146.3, 153.7, 154.7, 155.2, 155.6, 157.1, 165.4 (33C, Ar–C), 168.5, 168.7 (2C, 2C=O); MS (70 ev, %): m/z = 604 (M$^+$, 95%); Anal. Calc. for $C_{36}H_{24}N_6O_4$ (604.63): C, 71.51 (71.60); H, 4.00 (4.10); N, 13.90 (13.98).

4.28. Synthesis of 8-phenyl-6-(quinolin-2-yl)-11H,18H-pyrido [3',2':5,6] pyrimido [2,1-b:4,3-b'] diquinazoline-11,18-dione (16)

A blend consisting of compound **15** (6.04 g, 10 mmol) was mixed with glacial acetic acid (50 mL) and a small amount of concentrated sulfuric acid (1 mL). The mixture was then heated and stirred for 11–14 h under controlled conditions (monitored by thin-layer chromatography). After the reaction, the solution was allowed to cool to room temperature, poured over crushed ice and cold water, and neutralized with ammonia solution (NH$_4$OH). The resulting solid product was filtered, washed with water, dried, and finally crystallized from dioxane. This yielded yellowish crystals with a melting point greater than >350 °C (dec.) and a yield of (73%); IR (ν, cm^{-1}) KBr: 3072 (CH, aryl), 2937 (CH alkyl), 1690, 1685 (two C=O), 1625 (C=N), 1592 (C=C); ^{1}H NMR (DMSO-d_6, ppm) δ 7.04–8.20 (m, 19H, three phenyl, quinoline rings), 8.30 (s, 1H, pyridine);^{13}C NMR (DMSO-d_6) δ 112.3 (1C, CH, pyridine), 113.7, 113.8, 117.9, 121.3, 121.6, 121.8, 128.2, 128.4, 129.3, 130.1, 130.2, 130.3, 133.4, 136.5, 139.6144.4144.5, 144.6, 144.8, 151.6, 154.1, 154.3, 154.4, 154.5, 154.6, 160.1, 160.3, 160.4, 160.5, 160.6, 160.7 (33C, Ar–C), 170.2, 170.3 (2C, 2C=O); MS (70 ev, %): m/z = 568 (M$^+$, 91%); Anal. Calc. for $C_{36}H_{20}N_6O_2$ (568.60): C, 76.05 (76.15); H, 3.55 (3.50); N, 14.78 (14.71).

4.29. Pharmacological screening

4.29.1. Materials and methods for in-Vitro anti-proliferative activities
The research aimed to assess how certain compounds could prevent the growth of human breast adenocarcinoma cells (MCF-7) and human normal Retina pigmented epithelium cells (RPE-1) by using the 3-[4,5-dimethyl-2-(thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. These cell lines were obtained from ATCC located in (Rockville, MD, USA) [67,80–82]. The cells were grown in a sterile micro-plate with 96 wells, each containing 5 × 104 cells. The plate was kept at a temperature of 37 °C in RPMI-1640 medium, which was supplemented with (10%) heat-inactivated fetal bovine serum (FBS) and 100 U/mL of both penicillin and streptomycin. The environment inside the plate was maintained at a 5% CO$_2$ humidified atmosphere. After 24 h, the media was replaced with serum-free medium of (90 μL) per well. Then, a series of compounds or doxorubicin® (positive control) concentrations in DMSO were added in (10 μL) quantity. This mixture was kept for 48 h. The experiment involved adding MTT (40 μL of 2.5 mg/mL) to each well after the removal of media, followed by incubation for 4 h. In the final step, 200 μL of DMSO was added to dissolve the purple-colored formazan dye crystals, and the absorbance was measured at 590 nm using a Spectra Max® Paradigm® multi-mode microplate reader. The experiment was conducted in triplicate and repeated on three different days. The relative cell anti-proliferative activity was measured using the following formula: % cytotoxicity = (1-As/Ab) *100, whereas is the absorbance of each sample and Ab is the absorbance of the blank. Data was analyzed using the SPSS software program (version 20, SPSS Inc., Chicago, IL, USA) to determine each IC$_{50}$ value.

4.29.2. Materials and methods for in-Vitro antioxidant activity
This study aims to determine the antioxidant activity of each compound and standards such as Rutin and ascorbic acid. The activity will be measured by evaluating their radical scavenging effect on stable DPPH free radicals [67,68]. To conduct the experiment, 10 μl of each compound or standard with different concentrations were added to a 96-well microtiter plate. The plate already contained 90 μl of a 100 μM methanolic solution of DPPH. After adding the solutions, the plate was incubated in the dark at 37 °C for 30 min. Later, the absorbance of each solution was measured at 520 nm using an ELISA microplate reader. Additionally, blank samples containing the same amount of DMSO and DPPH solution were also prepared and measured to serve as a control. The experiment was carried out in triplicate. To calculate the radical scavenging activity of each compound, the following formula was used:

% Reduction of absorbance = [(AB − AA) / AB] × 100

At t = 30 min, the tested compound's absorbance (AA) and the blank sample's absorbance (AB) were measured. The concentration

of each compound required to scavenge 50% of DPPH (IC$_{50}$) was also determined [67,68].

4.29.3. Ethical Approval and Consent to Participate

No humans or animals were used in this study. However, all procedures were performed under the Medical Research Ethics Committee of Mansoura University, Faculty of Pharmacy, Department of Pharmacognosy, Egypt.

4.29.4. Human and animal Rights

No living beings were subjected to the study. The research was carried out *in vitro* in line with ethical standards.

4.29.5. Chemicals and drugs

Two types of human carcinoma cancer cell lines, MCF-7 and RPE-1 were obtained from ATCC (Rockville, MD, USA) and the National Cancer Institute, Cairo University, Cairo, Egypt. Furthermore, Doxorubicin, Rutin, Ascorbic acid, and DMSO were purchased from Sigma-Aldrich (Saint Louis, MO, USA).

5. Conclusions

The manuscript focuses on two important types of heterocyclic compounds: pyrido [2,3-*d*] pyrimidinone and quinoline/quinazoline rings. These compounds are found in many cells and neurotransmitters and play a key role in the formation of DNA and RNA in living organisms, including thymine, cytosine, and uracil. Quinoline alkaloids are classified into three types: 2-*n*-propylquinoline, chimanine-D, and chimanine-B. These alkaloids are present in plants from the families Moraceae, Annonaceae, and Rutaceae in Sri Lanka, and are extracted from the Bolivian plant Galipea longiflora Krause. Furthermore, we synthesized new heterocyclic compounds such as substituted-6-quinoline-pyridotriazolopyrimidinones (**5a-d**); 2-(methyl-sulfonyl)-(quinoline) pyrido-pyrimidinones (**6**); 2-substituted-5-(quinoline) pyridopyrimidinones (**7a, b**); substituted-pyrido [2,3-*d*]pyrimidines (**8, 10, 11, 13a-c, 14a,b, 15**); substituted-pyridopyrimido [2,1-*b*] quinazoline (**9**); substituted-pyrido [3,2-*e*]bis ([1,2,4]triazolo)pyrimidine (**12a, 12b**) and substituted-pyrido [3′, 2′:5,6]pyrimido [2,1-b:4,3-*b'*]diquinazoline-dione (**16**) derivatives. Molecular docking studies were conducted to investigate the binding of prepared compounds to amino acids such as Estrogen Receptor alpha, EGFR, and NADPH oxidase protein. In addition, the compounds were tested for their anticancer antioxidant activity *in vitro*. Based on the results of biological assays and previous research, we have concluded that the anticancer activities of specific compounds are attributed to the reaction between pyrido [2,3-*d*] pyrimidine derivatives. Among the compounds tested, **5a-d**, **9**, **12a-b**, and **16** showed promising anticancer activity; the same compounds could effectively treat a selected range of cancer cells in the future. Thus, we have identified them as promising anticancer agents.

Additional information

No additional information is available for this article.

Data availability statement

All data is included in the article, and a supplementary information data file is attached.

CRediT authorship contribution statement

Ameen Ali Abu-Hashem: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Othman Hakami:** Validation, Software, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Nasser Amri:** Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26735.

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