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Research article

Synthetic approaches for novel fused pyrimidine derivatives: Design, structural characterization, antiviral, antitumor, and molecular docking evaluation

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ABSTRACT

The goal of this work was to synthesize new compounds for anticancer evaluation as a trial to obtain new antitumor agents with higher activity and fewer side effects. Therefore, the precursor 2,2'-(1,4-phenylenebis (thiazole-4,2-diyl))bis (3-(dimethylamino)acrylonitrile) **(4)** was used to synthesize various azolopyrimidine derivatives connected to the thiazole moiety. Compounds **5**–**11**, including pyrazolopyrimidine, triazolopyrimidine, and others, were produced by reacting enaminonitrile **4** with different *N*-nucleophiles. Additionally, compounds **12**–**15**, such as isoxazole and pyrimidinethione derivatives, were obtained by reacting compound **4** with guanidine, hydrazine hydrate, hydroxylamine hydrochloride, and thiourea. Enaminonitrile **4** was also treated with barbituric acid, isoxazolone, and pyrazolone to yield pyranopyrimidine derivatives **18**–**20**. Moreover, enaminonitrile **4** reacts with *C*-nucleophiles namely ''acetylacetone, dimedone, 2-cyanomethylbenzothiazole, and 2-cyanomethylbenzimidazole'' to give pyrano derivatives **21**, **22** and fused pyridone derivatives **23** and **24**, respectively. The cytotoxic activity of **20** novel compounds against HSV-1, HIV-1, and various cancer cell lines was assessed, with compounds **5**, **7**, and **9** showing the strongest effects. Molecular docking studies further evaluated the binding affinity of these derivatives, with docking scores ranging from −7.8679 to −8.3013 kcal/mol. Several new azolopyrimidine derivatives linked to the thiazole moiety were effectively synthesized and assessed in the study, and they showed notable cytotoxic activity against HSV-1, HIV-1, and several cancer cell lines.

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1. Introduction

The study of heterocyclic compounds is important for both medical and organic chemistry. Most pharmaceuticals that are marketed contain heterocyclic motifs, which are essential to the medications' ability to produce the intended pharmacological effects. Furthermore, a variety of enhanced synthetic techniques have been used to synthesize these heterocycles [1]. Moreover, these compounds serve as an important intermediate for synthesizing various biologically significant fused heterocyclic compounds [2]. These compounds are present in a broad spectrum of drugs, antibiotics, vitamins, natural products, and numerous other biomolecules. They play important roles in biological processes. Furthermore, nitrogenous heterocyclic compounds have drawn a lot of interest and are found in a variety of agrochemicals, organic materials, medicines, and bioactive natural items [3].

Heterocyclic scaffolds known as nitrogenous bases are crucial to many aspects of biology. They form the main framework of numerous essential biomolecules. The fundamental building elements of molecules like DNA and RNA that convey genetic information are pyrimidine and purine pharmacophores. Additionally, they take part in a number of biological activities, such as cell signaling. Consequently, they were extensively used in the creation of various veterinary medications, agricultural goods, cardiovascular medications, and chemotherapy therapies [4]. Numerous analogues of purines and pyrimidines exhibit a wide range of biological activities, such as antagonistic actions on the serotonin 5-HT6 receptor, anti-arrhythmic, analgesic and inflammatory properties, antimicrobial, and protective properties against avian influenza virus (H5N1), herpes simplex virus type-1 (HSV-1), and hepatitis A virus (HAV) [5,6].

Cyclin-dependent kinase (CDK) inhibitors [7], anti-proliferative [8], anti-bacterial [9], antifungal [10], anti-viral agents [11], and anti-leishmanial [12] are just a few of the pharmacological characteristics displayed by pyrazolopyrimidines. Moreover, pyrazolopyrimidines have the following additional effects: depressants of the central nervous system [13], selective inhibitors of COX-1 and COX-2 [14], sedatives and antitrypanosomes [15], antagonists of serotonin 5-HT6 receptors [16], antagonists of corticotrophin releasing factor (CRF) 1 receptor [17], tuberculostatic [18], and agents for PET tumor imaging [19]. In the past, pyrazolopyrimidines were mostly described as antagonists of the adenosine receptor [20].

Pyrazolopyrimidine-derived compounds are synthesized and investigated as effective antineoplastic medicines among TKIs (tyrosine kinase inhibitors). In contrast to the imidazole moiety in purines, the pyrazole ring fused with the pyrimidine moiety creates the pyrazolopyrimidines [21]. Some pyrazolopyrimidine-marketed drugs such as allopurinol, zaleplon, indiplon [22], loredipl on [23] sildenafil [24], tisopurine [25] (Fig. 1).

Novel derivatives of pyrazolo [4,3-e]Triazolo [1,2,4]pyrimidines have anticancer properties and function as kinase inhibitors [26,27]. Tetrazole [28] and pyrimidine [29] moieties, exhibit a variety of biological activities including antimicrobial [30], antifungal [31], antimalarial [32], anticancer [33], antihypertensive [34], and anti-tuberculosis [35], are also well-explored as important substructure motifs of several medicinal molecules within such a scaffold. Furthermore, the pyran core structure is a well-known

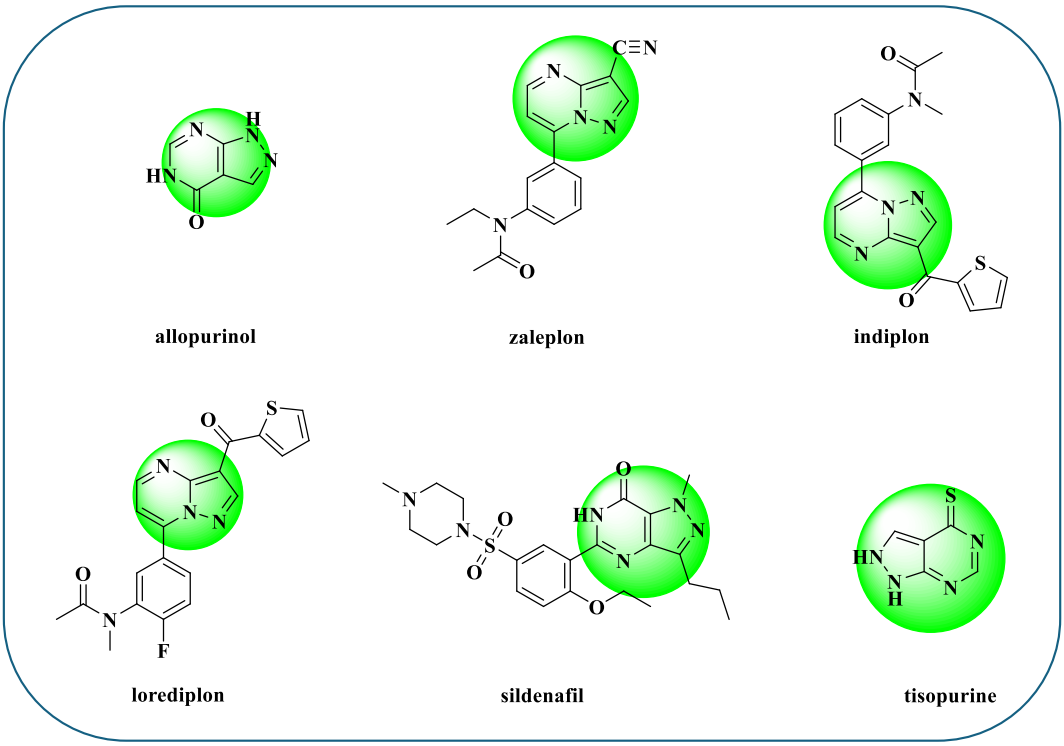


Fig. 1. Some of the marketed drugs containing pyrazolopyrimidine ring.

heterocyclic framework that is commonly present in a wide range of natural products as well as synthetic compounds with bioinspired properties [36]. Because of its wide range of pharmacological actions, it has a major functional role from the standpoint of medicinal chemistry [37]. Therefore, there is a renewed interest among synthetic organic chemists in the synthesis of 4-H pyran motifs.

Furthermore, thiazole derivatives are a fundamental scaffold that is present in a wide range of naturally occurring substances, including thiamine, alkaloids, steroids, flavones, and anabolics [38]. Because of their broad-spectrum action, such as their antibacterial, antifungal, antimalarial, antitubercular, and even anticancer activities (tiazofurin), thiazole derivatives are recognized as the most active class of chemicals. Numerous natural compounds contain thiazole moieties as crucial structural elements. Furthermore, thiazole-based compounds can be altered in several ways to produce brand-new compounds with potent anti-tumor capabilities [39]. Because of its many uses in the treatment of breast cancer, thiazoles have attracted a lot of attention. By preventing the angiogenesis of new blood vessels, thiazole inhibits the growth and spread of cancer cells [40a].

The incorporation of multi-heterocyclic systems into drug design is particularly advantageous due to the diverse range of biological activities exhibited by heterocyclic compounds, including anticancer, antiviral, and antimicrobial properties. Heterocyclic systems such as thiazoles, pyrimidines, and isoxazoles, commonly used in the synthesis of therapeutic agents, play a critical role in increasing drug efficacy due to their ability to interact with various biological targets. By combining these systems into a single molecule, researchers can exploit the synergistic effects of different pharmacophores, potentially leading to compounds with enhanced potency, selectivity, and improved pharmacokinetic profiles. This molecular diversity not only improves drug-target interactions but also broadens the spectrum of biological activity, making multi-heterocyclic hybrids highly desirable in the quest for new therapeutic agents. By incorporating multi-heterocyclic structures, the development of anticancer, antiviral, and antimicrobial agents can be significantly advanced, as evidenced by the successful synthesis of novel compounds with promising cytotoxic and docking results in the current research [40b,40c].

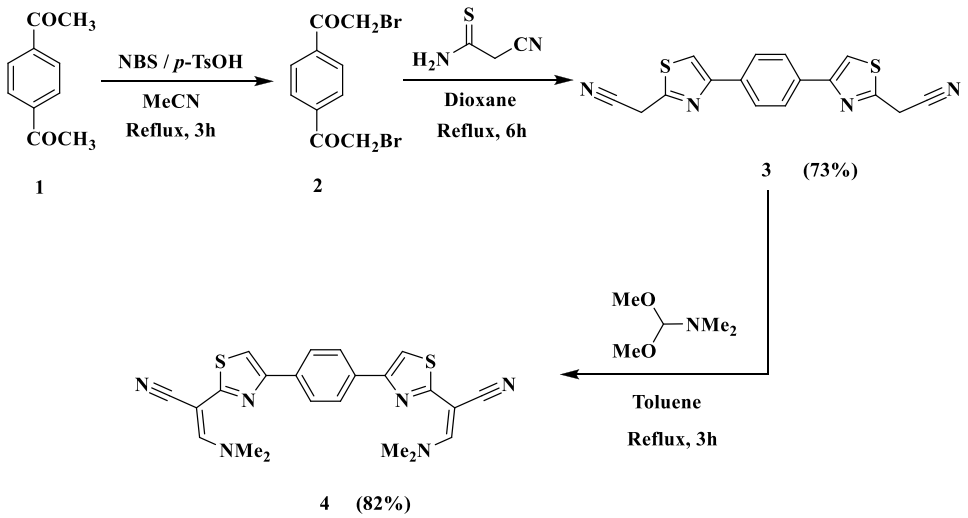
2. Results and discussion

2.1. The discussion of new synthesizes analyses

The published work [41] reported that 1,4-diacetylbenzene **(1)** was brominated in glacial acetic acid, resulting in the formation of the 1,4-dibromoacetyl derivative **(2)**. Upon refluxing in dioxane for 6 h, compound **2** reacted with two equivalents of 2-cyanothioacetamide to yield 2,2'-(1,4-phenylenebis (thiazole-4,2-diyl))diacetonitrile **(3)** (Scheme 1). Compound **3's** structure was verified by spectrum data and elemental analysis. Compound **3's** infrared spectra showed an absorption of the CN group at ν 2220 cm^{-1}. Two singlet signals, corresponding to the protons of the CH_2CN and thiazole CH_2, were seen in the ^1H NMR spectrum at δ 3.70 and 7.95 ppm, respectively.

An important starting point for the synthesis of many azolopyrimidine derivatives linked to the thiazole moiety was the bis(2-thiazolyl)acetonitrile **3**. Therefore, the corresponding 2,2'-(1,4-phenylenebis (thiazole-4,2-diyl))bis (3-(dimethylamino)acrylonitrile) **(4)** was obtained by refluxing compound **3** with *N,N*-dimethylformamide dimethyl acetal (DMF/DMA) in toluene for 3 h (Scheme 1). Analytical and spectral data were used to establish the structure of compound **4**, which was identified as the reaction result. An absorption band in the IR spectrum showed at ν 2196 cm^{-1}, which corresponds to the conjugated CN function. Two sharp singlet signals at δ 3.00 ppm were identified in the ^1H NMR spectrum as belonging to the *N,N*-dimethylamino protons. Two more singlet signals at δ 6.80 and 8.15 ppm were identified as the methine and thiazole proton, respectively.

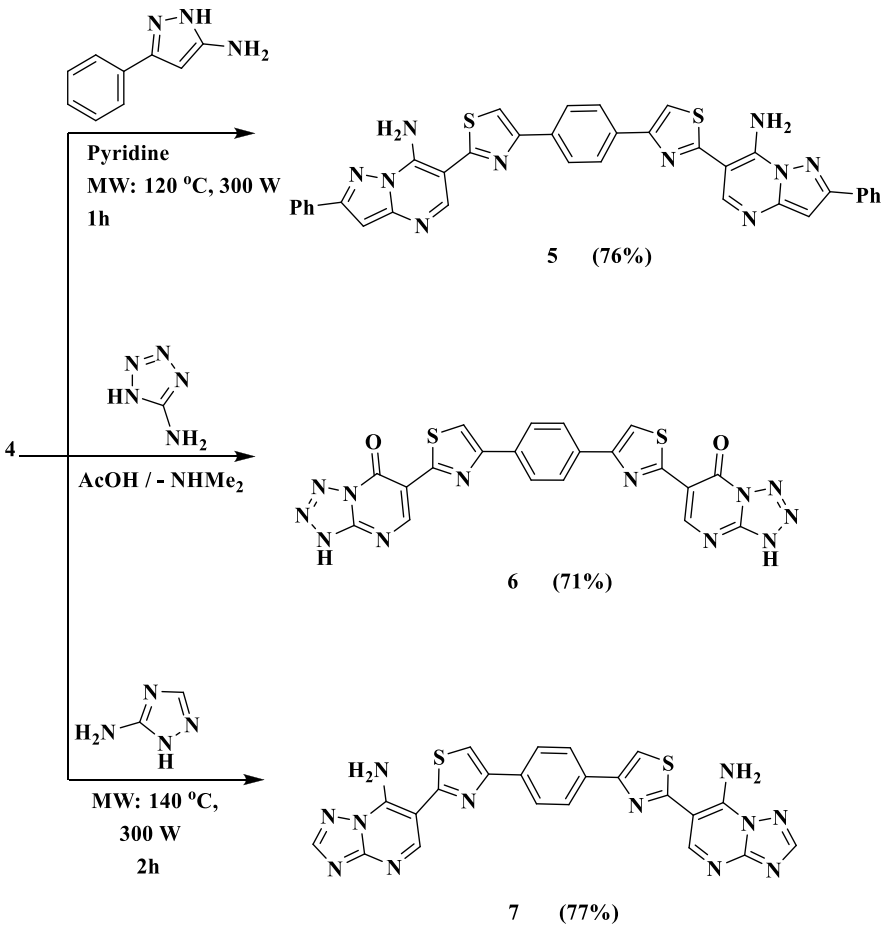
To obtain polyfunctionally substituted azoles, azines, and analogous fused systems linked to a thiazole moiety of potential



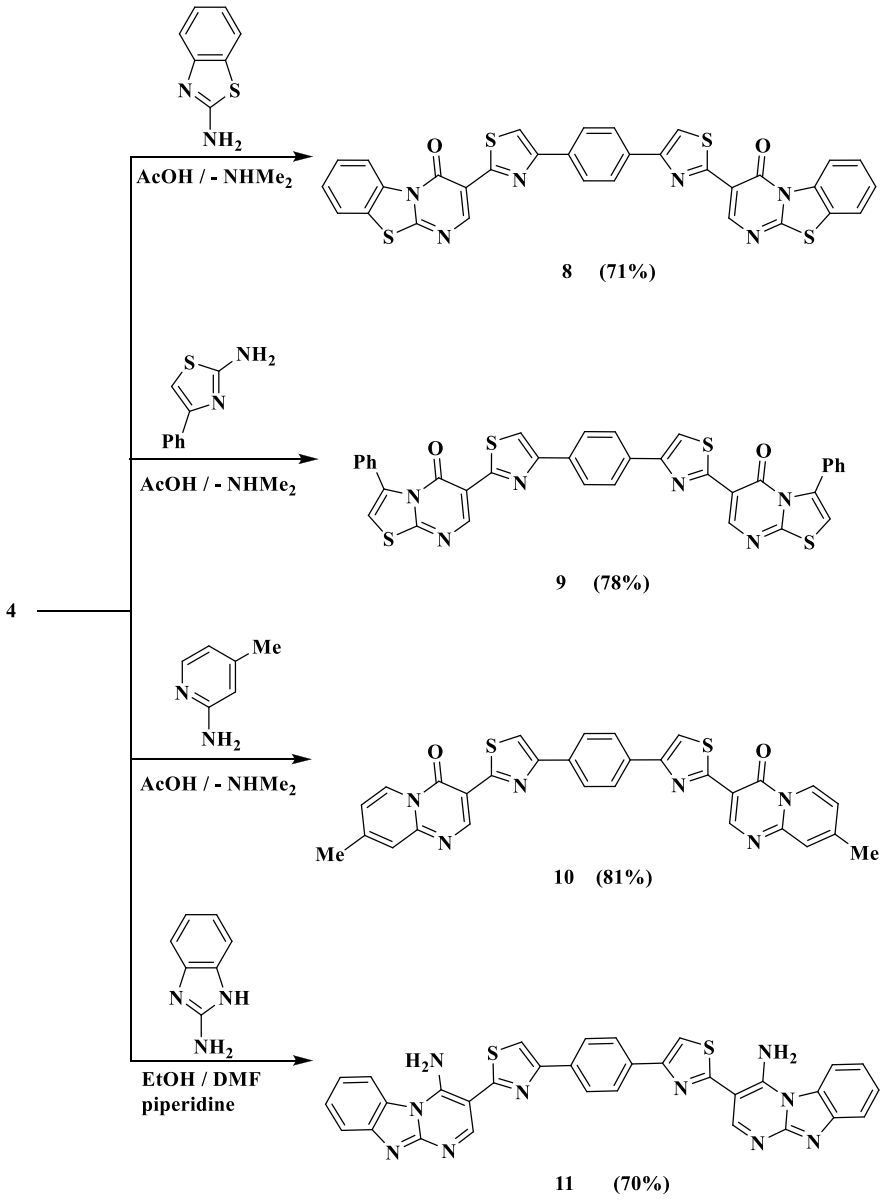
Scheme 1. Synthesis of enaminonitrile **4**.

pharmacological significance, the behavior of enaminonitrile **4** towards certain *N*-nucleophiles has been studied. Compound **4** reacted with three heteraryl amines: 5-aminopyrazole, 5-aminotetrazole, and 5-aminotriazole in refluxing glacial acetic acid to produce 6,6'-(1,4-phenylenebis (thiazole-4,2-diyl))bis (2-phenylpyrazolo [1,5-a]pyrimidin-7-amine) **(5)**, 6,6'-(1,4-phenylenebis (thiazole-4,2-diyl))bis (tetrazolo [1,5-a] pyrimidin-7(3H)-one) **(6)**, and 6,6'-(1,4-phenylenebis (thiazole-4,2-diyl))bis ([1,2,4] triazolo [1,5-a] pyrimidin-7-amine) **(7)**, respectively (Scheme 2). While compound **6's** IR spectrum revealed absorption bands at ν 3220, 1680, and 1610 cm^{-1} attributable to NH, CO, and C=N groups, respectively, the IR spectra of compounds **5** and **7** showed no bands around the region at ν 2220 cm^{-1} due to CN function and instead showed absorption bands at ν 3350 and 1620 cm^{-1} corresponding to NH_2 and C=N groups, respectively. Compound **5's** ^{1}H NMR analysis produced four distinct singlet signals at δ 6.71, 6.90, 7.78, and 8.79 ppm, which are associated with the pyrazole-H proton, NH_2, pyrimidine-H proton, and thiazole-H proton, in that order. In contrast, compound **6's** ^{1}H NMR revealed three singlet signals at δ 8.28, 8.99, and 9.50 ppm, which were attributed to the thiazole-H proton, pyrimidine-H proton, and NH, respectively. Four singlet signals were seen at δ 6.76, 7.89, 8.69, and 8.88 ppm in compound **7's** ^{1}H NMR, which are attributed to NH_2, pyrimidine-H proton, thiazole-H proton, and triazole-H proton, in that order.

The synthesis of 3,3'-(1,4-phenylenebis (thiazole-4,2-diyl))bis (4H-benzo [4,5]thiazolo [3,2-a]pyrimidin-4-one) **(8)** and 6,6'-(1,4-phenylenebis (thiazole-4,2-diyl))bis (3-phenyl-5H-thiazolo [3,2-a]pyrimidin-5-one) **(9)** was of interest due to the growing biological significance of fused thiazoles, in particular thiazolo [3,2-a]pyrimidines [42,43]. A system like this is regarded as a thia-analogue of adenine and guanine, the natural purine bases. In order to obtain compounds **8** and **9**, respectively, enaminonitrile **4** was refluxed with 2-aminobenzothiazole or 2-aminothiazole in acetic acid (Scheme 3). Compounds **8** and **9's** infrared spectra revealed bands at ν 1685 and 1615 cm^{-1} owing to amidic carbonyl and C=N functionalities, and a lack of absorption band at ν 2220 cm^{-1}, which corresponded to the conjugated CN group. Compound **8's** ^{1}H NMR spectrum showed two singlet signals, unique for the thiazole-H proton and pyrimidine-H proton, at δ 8.11 and 8.40 ppm, respectively. Compound **9's** ^{1}H NMR spectra revealed three singlet signals at δ 6.30, 8.20, and 8.49 ppm, which corresponded to the proton of the fused thiazole-H, thiazole-H ring, and pyrimidine-H, respectively. Furthermore, in refluxing acetic acid, enaminonitrile **4** reacted with a 2-aminopyridine derivative to give 3,3'-(1,4-phenylenebis (thiazole-4,2-diyl))bis (8-methyl-4H-pyrido [1,2-a]pyrimidin-4-one) **(10)** (Scheme 3). Compound **10's** ^{1}H NMR spectrum revealed two doublets at δ 7.09 and 8.82 ppm specific to protons on the pyridine ring, two singlet signals at δ 8.29 and 9.06 ppm corresponding to the protons on the thiazole and pyrimidine rings, respectively, and a singlet signal at δ 2.25 ppm characteristic of the methyl proton.



Scheme 2. Reaction of enaminonitrile **4** with some *N*-nucleophiles.

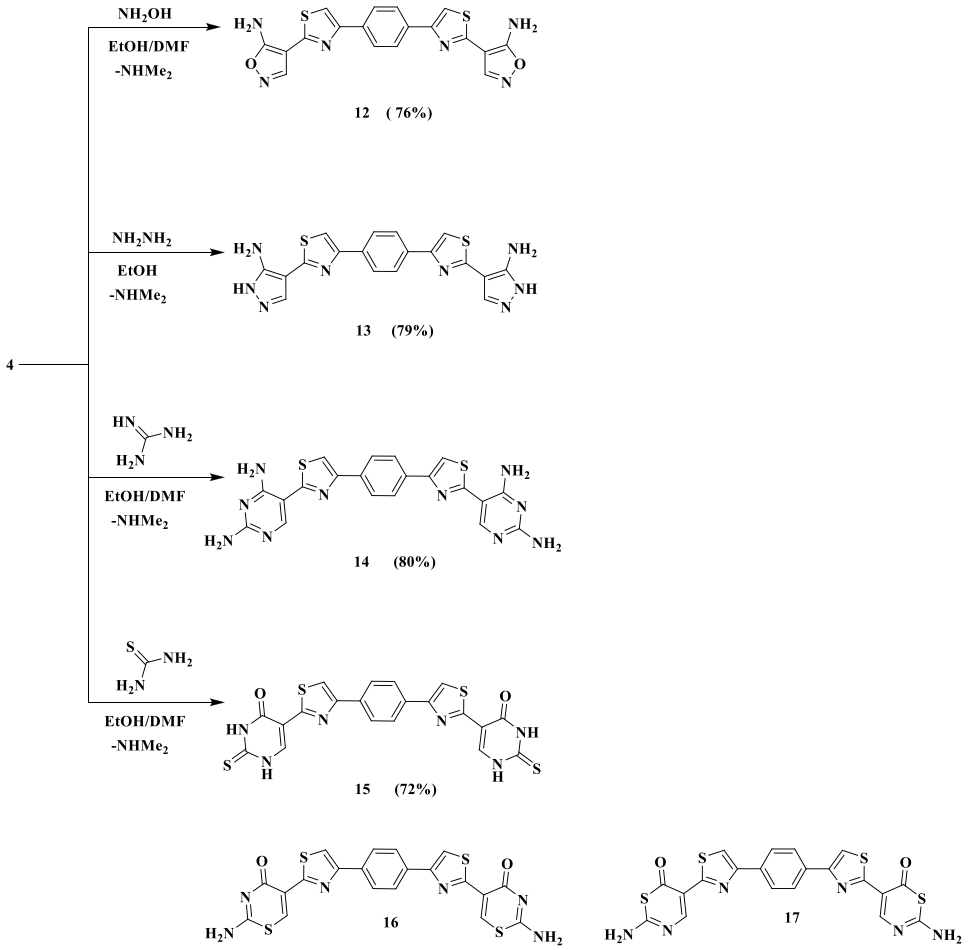


Scheme 3. Synthesis of some new fused pyrimidine derivatives **8**–**11**.

Furthermore, employing a catalytic quantity of piperidine, the reaction of enaminonitrile **4** with 2-aminobenzimidazole in ethanol produced 3,3'-(1,4-phenylenebis (thiazole-4,2-diyl)) bis(benzo [4,5]imidazo [1,2-a]pyrimidin-4-amine) **(11)** (Scheme 3). Compound **11's** structure was verified by spectrum data and elemental analysis. Compound **11's** IR spectrum showed absorption bands at ν 3450 and 3320 cm^{-1} corresponding to NH_2, and its ^1H NMR spectrum showed D_2O-exchangeable signal at δ 6.65 ppm, indicating the presence of an amino group.

Also, the reaction of compound **4** with hydroxyl amine hydrochloride in refluxing ethanol/dimethylformamide (1:1) with anhydrous potassium carbonate was investigated. The result was 4,4'-(1,4-phenylenebis (thiazole-4,2-diyl))bis (isoxazol-5-amine) **(12)** (Scheme 4). Compound **12's** structure and the separated product's spectrum data were only partially compatible. The infrared spectra showed absorption bands at ν 3400–3350, and 1620 cm^{-1}, corresponding to NH_2 and C=N functions, respectively, and showed the absence of an absorption band corresponding to a conjugated CN function. The ^1H NMR spectra showed a singlet signal at δ 8.40 ppm, which is specific for aromatic protons, along with three characteristic signals at δ 6.35, 8.07, and 8.77 ppm, which were assigned to the NH_2, isoxazole-H_3, and thiazole-H_5 protons, respectively.

Enaminonitrile **4** was heated with hydrazine hydrate in ethanol, yielding aminopyrazole **13** as the sole product (Scheme 4). Inspection of the ^1H NMR spectra allowed for the confirmation of compound **13's** structure. The pyrazole-H_3 proton in this pyrazole derivative was identified at δ 7.98 ppm as a singlet, whereas the NH_2 and NH protons were detected at δ 5.30 and 12.70 ppm,



Scheme 4. Synthesis of isoxazole, pyrazole, amino pyrimidine, and pyrimidine thione **12**–**15**.

respectively. Additionally, a characteristic signal for the thiazole proton was detected at δ 8.69 ppm. Notably, no signals corresponding to the tautomeric 3-aminopyrazole were observed in the ^{1}H NMR spectrum, which would typically display the pyrazole-H_5 as a doublet.

Similarly, 5,5'-(1,4-phenylenebis (thiazole-4,2-diyl))bis (pyrimidine-2,4-diamine) **(14)** was synthesized when enaminonitrile **4** reacted with guanidine in a mixture of ethanol and DMF (2:1) containing anhydrous potassium carbonate under reflux (Scheme 4). Compound **14's** infrared spectra showed bands at ν 3410-3250 cm^{-1} that corresponded to the $2NH_2$ groups and at 1615 cm^{-1} that was associated with the C=N group. The pyrimidine-H_6 proton was responsible for a singlet signal at δ 8.16 ppm in the ^{1}H NMR spectrum, while the thiazole-H_5 proton was responsible for a singlet signal at δ 8.82 ppm. The two NH_2 protons were represented by the singlet signal at δ 6.46 ppm in the ^{1}H NMR spectrum.

It is thought that compound **4's** activated double bond is subjected to a Michael-type addition by the amino groups of hydroxylamine, hydrazine, and guanidine, creating a non-isolable Michael adduct intermediate. Intramolecular cyclization of this intermediate is easily accompanied by the removal of dimethylamine molecules, resulting in the creation of the target compounds, which are suggested to be compounds **12**, **13**, and **14**.

Additionally, the site selectivity in the cycloaddition of enaminonitrile **4** with several nitrogen ambident nucleophiles was investigated. As a result, three isomeric cycloadducts, **15**, **16**, and **17**, were considered possible for the reaction of compound **4** with thiourea in refluxing ethanol containing a catalytic amount of piperidine. On the other hand, Scheme 4's TLC analysis revealed the formation of a single product. Pyrimidinethione **15** was determined to be the reaction product using elemental analysis and spectrum data analysis. Compound **15's** infrared spectra indicated absorption bands at ν 3395–3162, 1680, 1625, and 1279 cm^{-1}, which are indicative of two NH, amidic C=O, C=N, and C=S functions, respectively, instead of an absorption band corresponding to a CN function. Instead of the predicted signal for structures **16** or **17**, the ^{1}H NMR spectrum had a singlet signal at δ 7.15 ppm that attributed to the pyrimidine-H_6 proton. Two singlet signals (corresponding to four NH protons) were also detected at δ 7.50 and 12.60 ppm, in addition, aromatic protons appeared at δ 8.59 ppm. The structure was further validated by the ^{13}C NMR spectra, which identified 20 carbon types. The carbonyl carbons of cyclic amide and C=S were revealed by the major signals at δ 165.5 and 175.7 ppm, respectively.

It has also been studied the reaction of enaminonitrile **4** with an active methylene group of heterocyclic compounds. The compound

6,6'-(1,4-phenylenebis (thiazole-4,2-diyl))bis (2H-pyrano [2,3-d]pyrimidine-2,4,7(1H, 3H)-trione) **(18)** was produced by cycloaddition of **4** with barbituric acid in glacial acetic acid (Scheme 5). Based on its elemental analyses and spectral data, compound **18's** structure was determined for the reaction product (*c.f.* experimental section).

Furthermore, the reaction of 4 with 3-phenylisoxazol-5(4H)-one in refluxing glacial acetic acid also yielded 5,5'-(1,4-phenylenebis (thiazole-4,2-diyl))bis (3-phenyl-6H-pyrano [3,2-d] isoxazole-6-one) **(19)**. To elucidate its chemical structure, **19's** spectrum data and elemental analysis were employed. The IR spectra revealed an absorption band at ν 1730, 1615, and 1580 cm^{-1} for the pyran CO, C=N, and C=C, respectively, and were free of a nitrile function. In its ^{1}H NMR spectra, a fused pyran (H_4) proton singlet at δ 7.31 ppm and a thiazole-specific singlet signal at δ 8.10 ppm were identified.

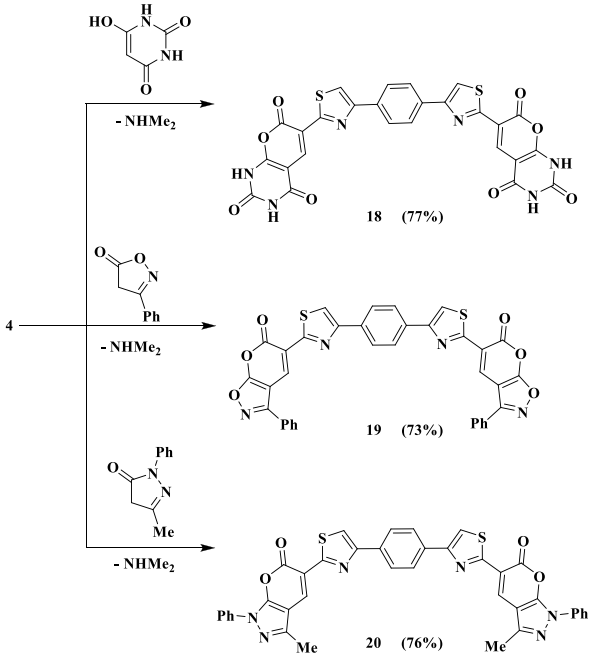
The equivalent 5,5'-(1,4-phenylenebis (thiazole-4,2-diyl))bis (3-methyl-1-phenylpyrano [2,3-c]pyrazol-6(1H)-one) **(20)** was generated by an analogous treatment of **4** with 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one under the same reaction conditions (Scheme 5). Their elemental research and spectra made the structure of product **20** more clear. Compound **20's** infrared spectrum showed that there was no absorption band for the nitrile function and that there were absorption bands at ν 1720 and 1615 cm^{-1}, which are indicative of the pyran CO and C=N functions, respectively. Three singlet signals at δ 2.30, 7.49, and 8.02 ppm in its ^{1}H NMR spectra were identified as belonging to the methyl proton, pyran-H, and thiazole-H protons, respectively.

Similarly, it is proposed that the active methylene group of heterocycles is added by Michael to compound **4's** activated double bond to initiate the production of products **19** and **20** and non-isolable intermediates. After that, these intermediates go through tandem cyclization, deamination, hydrolysis, and the removal of dimethylamine to produce the end products, **19** and **20**.

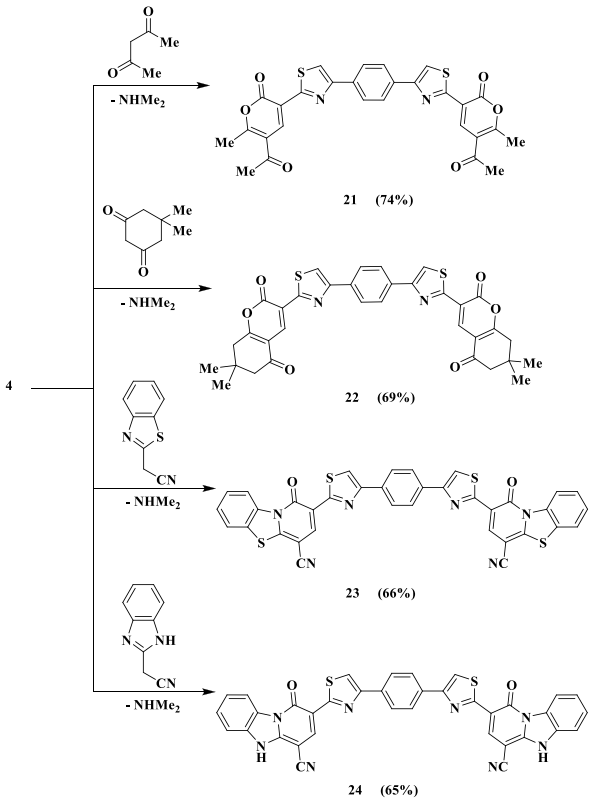
In continuation of this work was the study of enaminonitrile **4's** reactivity with several *C*-nucleophiles that had an active methylene group. The result of this reaction was 3,3'-(1,4-phenylenebis (thiazole-4,2-diyl))bis (5-acetyl-6-methyl-2H-pyran-2-one) **(21)** when **4** was reacted with acetylacetone in glacial acetic acid under reflux (Scheme 6). Accurate elemental analysis and spectrum data were used to infer compound **21's** structure. Carbonyl function-specific absorption bands had appeared in the IR spectra at ν 1725 and 1710 cm^{-1}. Compound **21** is thought to be formed when a dimethylamine molecule is lost and the active methylene group of acetylacetone is added to the activated double bond in enaminonitrile **4**. Compound **21** is produced *via* intramolecular cyclization of a non-isolable acyclic intermediate that is formed because of this process.

Next, as a potential simple synthesis pathway to obtain coumarin analogues, the reactivity of enaminonitrile **4** toward dimedone was examined. A single isolable product, 3,3'-(1,4-phenylenebis (thiazole-4,2-diyl))bis (7,7-dimethyl-7,8-dihydro-2H-chromene-2,5 (6H)-dione) **(22)** was therefore produced by heating **4** with dimedone in glacial acetic acid (Scheme 6). Compound **22's** IR spectra, which showed absorption bands at ν 1715 and 1690 cm^{-1}, corresponding to pyran carbonyl (CO) and cyclic carbonyl (CO) functions, respectively, were used to determine the compound's structure. Five singlet signals were seen at δ 1.10, 1.76, 2.10, 8.05, and 8.39 ppm in the ^{1}H NMR spectrum. These signals were ascribed to the two geminal methyl groups, which are chromene (H_8), chromene (H_6), chromene (H_4), and thiazole-H protons, in that order.

Enaminonitrile **4** reacts with heterocyclic compounds that have an active methylene group connected at the α-position relative to the nitrogen atom of the ring, providing a straightforward synthetic pathway to polyfunctionally substituted fused pyridine derivatives. To develop fused pyridone derivatives **23** and **24**, we examined the reactivity of enaminonitrile **4** towards 2-(benzothiazol-2-



Scheme 5. Reaction of enaminonitrile **4** with some active methylene heterocyclic compounds.



Scheme 6. Reaction of enaminonitrile **4** with some *C*-nucleophiles.

yl)acetonitrile and 2-(benzimidazol-2-yl)acetonitrile in this context.

Furthermore, by heating enaminonitrile **4** with 2-cyanomethylbenzothiazole in refluxing glacial acetic acid, 2,2'-(1,4-phenylenebis (thiazole-4,2-diyl))bis (1-oxo-1H-benzo [4,5]thiazolo [3,2-a]pyridine-4-carbonitrile) **(23)** was formed (Scheme 6). Compound **23** exhibited distinct absorption bands at ν 1620, 1685, and 2219 cm^{-1} in its infrared spectra. These bands corresponded to the C=N group, two amidic C=O groups, and the CN group, respectively. The ^{1}H NMR spectra showed an aromatic multiplet in the δ 7.87–8.05 ppm range, a thiazole-H proton-attributed singlet at δ 8.49 ppm, and a singlet at δ 7.70 ppm caused by the fused pyridine (H_3) proton.

On the basis of elemental analysis and spectral data (*c.f.* experimental part), it was determined that the single product obtained from compound **4's** reaction with 2-(1H-benzimidazol-2-yl)acetonitrile in refluxing acetic acid was 2,2'-(1,4-phenylenebis (thiazole-4,2-diyl))bis (1-oxo-1,5-dihydrobenzo [4,5]imidazo [1,2-a]pyridine-4-carbonitrile) **(24)** (Scheme 6).

A tenable process that starts with the nucleophilic addition of the active methylene group from 2-(benzothiazol-2-yl) and 2-(benzimidazol-2-yl) acetonitrile to the β-carbon of enaminonitrile **4** can account to produce compounds **23** and **24**. The ring nitrogen atom is subsequently nucleophilically added to the nitrile group of these intermediates, causing *in situ* intramolecular cyclization. Hydrolysis and the removal of a dimethylamine molecule come next in this process, which finally results in the creation of the target products, **23** and **24**.

2.2. Biological screening

2.2.1. Antiviral activity

Pandemics of disease in humans and animals can be brought on by viruses that spread quickly and have large infection rates. Despite the recent appearance of novel virus types and previously unidentified viruses, viral infection continues to be a significant contributor to health issues in humans. Furthermore, the primary targets of the antiviral drugs now on the market are the important viral enzymes involved in the reproduction process. For a variety of viral infections, combination therapy with medications with various mechanisms of action and resistance profiles may be helpful. The development of innovative medicines possessing wide antiviral activity and unique antiviral mechanisms is crucial. To do this, 20 novel heterocyclic moieties connected to thiazole ring compounds **3**–**15** and **18**–**24** were screened and assessed for their ability to inhibit the growth of HSV-1 and HIV-1 *in vitro*. Positive control for HSV-1 cultured on Vero African green monkey kidney cells was the antiviral antimitotic antibiotic Aphidicolin [44]. Monolayers of labeled Vero cells treated with HSV-1 that are moderately confluent and unaffected are considered to exhibit antiviral activity. Vero cell culture was used to test the novel compounds' cytotoxicity [45]. The concentration that resulted in a roughly 50 % decrease in cell viability or proliferation was used to evaluate cytotoxicity. To assess the selected drugs, an enhanced plaque reduction

assay for antiviral activity was employed. Table 1 displays the results of the 20 compounds that were tested. Compounds **7**, **5**, and **9** showed marginal activity, reducing the number of plaques by 49, 45, and 41 %, respectively, at an effective concentration (EC_{50}) of 0.03, 0.05, and 0.09 μM/L, with cytotoxicity concentration (CC_{50} = 0.03, 0.09, and 0.07 μM/L, respectively). Compounds **8**, **10**, **11**, **6**, and **14** on the other hand, demonstrated a modest level of efficacy against HSV-1. The produced compounds exhibited varying degrees of cytotoxicity, according to the obtained data.

One of the factors contributing to the spread and progression of acquired immunodeficiency syndrome (AIDS) is HIV-1. AIDS is still one of the most serious global health issues, ranking as the fourth greatest cause of mortality globally and the primary cause of death in Africa [46]. Thus, this should be taken into consideration. Consequently, drugs operating at any step of the virus reproductive cycle are detected by the process employed to assess the *anti*-HIV-1 potency [47]. HIV-1 kills T4 cells as part of the experiment. Chemicals that obstruct viral activity will shield cells from being cytolyzed. The cytotoxic effect (CC_{50}) of the investigated compounds on uninfected cultures was compared with the median effective concentration (EC_{50}) of the compounds utilizing infected cells. Test chemicals were not used in the incubation of either the infected or uninfected cultures as controls. Cultures treated with azidovudine or azidothymidine (AZT) were also employed as positive controls. The *in vitro* evaluation against HIV-1 showed that some of the tested drugs exhibited a strong inhibitory effect whereas other compounds indicated moderate or no activity, based on the collected data shown in Table 2.

As a result, although compounds **8**, **10**, **11**, **6**, and **14** show moderate action against HIV-1, compounds **7**, **5**, and **9** have the strongest cytotoxic effect. Ultimately, the least amount of activity was shown by the remaining compounds.

2.2.2. Antitumor activity

Over six million people worldwide lose their lives to cancer each year, making it the leading cause of death [48]. The prudent move toward the ultimate objective of cancer control is prevention [49]. In contemporary medicine, there are numerous approaches to treating cancer. These consist of surgery, radiation, and chemotherapy. Nowadays, chemotherapy is thought to be the most successful cancer treatment strategy. It makes theoretical sense to intervene with chemopreventive medications earlier in the carcinogenesis process rather than using chemotherapeutic drugs to try and destroy tumors that have already grown. However, the majority of normal cells are adversely affected by most cancer chemotherapeutic drugs [50]. Finding novel anticancer chemicals with lower toxicity to host cells is therefore crucial [51,52]. Heterocyclic compound utilization was crucial to the fight against cancer and its control efforts. In order to create powerful and targeted medications, pharmacophores are frequently assembled on heterocyclic molecules as scaffolds. This is particularly true for heterocyclic compounds with five members on their ring [53], which are the building blocks of many molecules with an intriguing variety of biological activities. The goal of this research was to synthesize new compounds for anticancer evaluation as a trial to obtain new antitumor agents with higher activity and fewer side effects, taking into consideration all of the previously given information. In this study, compounds with various heterocyclic moieties connected to the thiazole ring were chosen, and their effects on the growth of hepatoma (HepG2), lung fibroblast (WI-38), African green monkey kidney (VERO), and human breast adenocarcinoma (MCF-7) cell lines were assessed and screened *in vitro* as growth inhibitors using standard MTT method. These results were compared to the established anticancer drug, 5-fluorouracil (*5-Fu*), as well as a trial aimed at finding more potent and less harmful agents. The concentration of the compounds that led to a 50 % reduction in cell growth was utilized to express the results.

Table 1
The cytotoxicity, anti-HSV-1 activities of compounds **3**–**15** and **18**–**24** and the antiviral antibiotic aphidicolin.

Compounds	Reduction in number of plaques, %	EC_{50} μM/L	Cytotoxicity (CC_{50})[a]
3	B	C	0.18
4	31	0.15	0.15
5	45	0.05	0.09
6	35	0.09	0.08
7	49	0.03	0.03
8	40	0.10	0.15
9	41	0.09	0.07
10	38	0.08	0.08
11	38	0.12	0.11
12	24	0.14	0.1
13	21	0.10	0.18
14	20	0.90	0.19
15	B	C	0.24
18	26	0.31	0.125
19	B	0.15	0.198
20	12	0.18	0.21
21	12	0.23	0.23
22	B	0.24	0.23
23	11	C	<1.50
24	9	C	<1.70
Aphidicolin	100	0.02	0.85

b B: 0 % reduction in the number of viral plaques.

c C: inactive compound.

[a] CC_{50}: the concentration of drug that caused 50 % loss of the monolayer present around the plaques.

Table 2
The cytotoxicity, anti-HIV-1 activities of compounds **3**–**15** and **18**–**24** and the antiviral drug Zidovudine (AZT).

Compounds	Cytotoxicity (CC_{50}), μM/L[a]	Effective concentration (EC_{50}), μM/L[b]	SI (CC_{50}/EC_{50})[c]
3	C	<100	C
4	25.50	<100	<0.25
5	<100	0.85	<100
6	55	<100	<0.55
7	<100	0.81	<100
8	69	<100	<0.69
9	<85	0.87	<97.70
10	60	<100	<0.60
11	65	<100	<0.65
12	70	<100	<0.70
13	70	<100	<0.70
14	60	<100	<0.60
15	62	<100	<0.62
18	50	<100	<0.50
19	45	<100	<0.45
20	35	<100	<0.35
21	25	<100	<0.25
22	30	<100	<0.30
23	30	<100	<0.30
24	23.50	<100	<0.23
AZT	35.60	0.70	50.86

[a] CC_{50}: 50 % inhibitory concentration (the molar concentration of the drug that caused 50 % inhibition of cell growth).
[b] 50 % effective concentration (the molar concentration of the drug that caused 50 % protection against HIV-1 cytopathic effect); C: inactive compound.
[c] SI: selectivity index: ratio CC_{50}/EC_{50}.

The obtained results in Table 3 indicates that certain compounds underwent a strong inhibitory activity in the *in vitro* evaluation, whereas other compounds exhibited moderate or no activity.

Consequently, the most effective cytotoxic action against HepG2 cell lines is exhibited by compounds **7**, **5**, and **9**. Compounds **8**, **10**, **11**, **6**, and **14** exhibit reasonable efficacy against HepG2 cell lines, in contrast. The remaining substances, in the end, showed the least amount of activity against HepG2 cell lines.

Furthermore, compounds **8**, **10**, **11**, **6**, and **14** demonstrated moderate cytotoxic effects against the WI-38 cell line, whereas compounds **7**, **5**, and **9** demonstrated the highest cytotoxic activity. Compounds **21**–**24**, on the other hand, showed less action against

Table 3
In vitro antitumor activities of tested compounds on different cell lines.

Compounds	IC_{50} (μM)			
	HepG2	WI-38	VERO	MCF-7
3	130.61	242.64	183.34	136.78
4	83.52	179.42	129.94	168.99
5	18.76	34.05	18.76	24.68
6	40.05	89.83	74.48	88.62
7	19.58	39.36	18.33	28.00
8	21.86	51.35	29.62	40.83
9	18.65	40.64	23.11	30.45
10	27.28	69.56	46.78	52.45
11	26.12	66.23	54.41	69.32
12	69.38	154.75	119.01	118.98
13	81.69	181.08	129.89	153.26
14	58.62	119.53	93.82	100.31
15	89.24	161.11	143.19	153.68
18	79.92	134.91	126.90	135.26
19	78.16	126.01	117.46	125.41
20	99.88	124.44	117.25	120.83
21	146.16	161.97	154.42	161.99
22	128.23	142.07	142.47	141.83
23	117.63	130.06	145.25	132.28
24	126.17	139.90	156.53	141.39
5-Fu	66.11	24.60	49.97	17.68
P-value	**	**	**	**
L.S.D 5 %	0.576	2.47	8.24	4.94

IC_{50} (μM): 1–10 (very strong), 11–25 (strong), 26–50 (moderate), 51–100 (weak), 100–200 (very weak), 200 (noncytotoxicity), 5-Flu = 5-fluorouracil is drug reference.

WI-38 cell lines. Compounds **7**, **5**, and **9** additionally show a wide range of action against MCF-7 cell lines. Compounds **8**, **10**, **11**, **6**, and **14** also demonstrated varying degrees of cytotoxicity against MCF-7 cell lines; the remaining compounds demonstrated the least amount of cytotoxicity. These results were in line with those of the experiments conducted on VERO cell lines.

2.3. Structure-activity relationship

Adenine (A), thymine (T), guanine (GUA), and cytosine (C) are the four types of nucleotides that make up DNA. While thymine and adenine are almost coupled, cytosine and guanine are coupled *via* hydrogen bonds [54,55]. When examining the cytotoxicity of synthetic compounds tested on various cell lines, two variables might be deemed reliable:

1) the creation of an intramolecular hydrogen bond with any DNA nucleotide.
2) The positive charge on the tested compounds and the negative charge on the cell wall are attracted to one other.

The following observations were made regarding the SAR:

- It was noticed that compound **7** has a more potent activity. The [1,2,4]triazolo [1,5-a]pyrimidine core is known for its significant biological activity, including anticancer properties. The presence of an amine group at the 7-position enhances hydrogen bonding interactions with biological targets, potentially increasing binding affinity and activity. The thiazole ring also contributes to biological activity due to its electronic properties and ability to interact with various enzymes and receptors.
- Compound **5** also has high cytotoxic activity against all the tested cell lines due to the fact that the pyrazolo [1,5-a]pyrimidine core is associated with diverse biological activities, including anticancer effects. The phenyl group can enhance π-π interactions with biological targets, potentially improving binding affinity and efficacy. The amine group at the 7-position and the thiazole ring contribute to additional hydrogen bonding and interactions.
- For compound **9**, the fused thiazolo [3,2-a]pyrimidine core is known for its biological activity. The phenyl group can enhance binding interactions through π-π interactions. The thiazole ring contributes to overall activity by interacting with biological targets.
- Moreover, compound **8** showed that the fused heterocyclic benzo [4,5]thiazolo [3,2-a]pyrimidine system is associated with potential anticancer activity. The thiazole ring and ketone group can enhance interactions with biological targets.
- While in compound **10**, the pyrido [1,2-a]pyrimidine core is known for its biological activity, including anticancer properties. The methyl group may influence the compound's solubility and binding affinity. The thiazole ring contributes to overall activity.
- Additionally, compound **11** the imidazo [1,2-a]pyrimidine core is associated with significant biological activity. The amine group at the 4-position can form hydrogen bonds, enhancing interactions with biological targets. The thiazole ring further contributes to activity.
- On the other hand, in compound **6**, the tetrazolo [1,5-a]pyrimidine core is associated with potential biological activity. The ketone group at the 7-position may influence hydrogen bonding and interactions with biological targets. The thiazole ring contributes to overall activity.
- While compound **14** showed that, the diamine substituents at the 2,4-positions enhance hydrogen bonding potential. The thiazole ring contributes to interactions with biological targets.
- In compound **12**, the isoxazole ring can enhance biological activity through electronic interactions. The thiazole ring and amine group contribute to binding affinity and hydrogen bonding.
- For compound **13**, the pyrazole ring with a thiazole ring can enhance interactions with biological targets. The amine group at the 5-position contributes to hydrogen bonding potential.
- For compound **4**, the dimethylamino group and thiazole ring can enhance electronic interactions with biological targets. The acrylonitrile group may contribute to overall activity.
- For compound **3**, a simple structure with a thiazole ring that contributes to biological activity. The nitrile group may influence electronic interactions.
- On the other hand, in compound **15**, the thioxo group and thiazole ring contribute to potential biological activity. The pyrimidine core is associated with various biological activities.
- While compound **18**, the pyrano [2,3-d]pyrimidine core with thiazole ring may have moderate activity. The trione groups can influence hydrogen bonding and interactions.
- Moreover, in compound **19**, isoxazole and pyrano systems with a phenyl group can contribute to moderate activity. The thiazole ring enhances interactions with biological targets.
- On the other hand, compound **20** showed that, pyrano-pyrazole system with a phenyl group for moderate activity. The thiazole ring contributes to biological interactions.
- While in compound **21**, pyran-2-one system with acetyl and methyl groups for moderate activity. The thiazole ring contributes to interactions.
- Additionally, compound **22** showed that, a chromene-dione system with thiazole ring for moderate activity. The dimethyl groups influence electronic interactions.
- While in compound **23**, benzo [4,5]thiazolo [3,2-a]pyridine core with a nitrile group for moderate activity. The thiazole ring contributes to biological interactions.
- Finally, compound **24** showed that, benzo [4,5]imidazo [1,2-a]pyridine core with a nitrile group for moderate activity. The thiazole ring contributes to interactions.

- For compounds **21**–**24**, the lower activity may be attributed to the large size of the heterocyclic ring, also fused heterocyclic ring decreases the activity.

Generally, any compound that contains NH_2, NH, or SH can attach itself to the unsaturated moiety of DNA or harm DNA through

Table 4
Statistical study of the newly synthesized compounds.

R	T	HepG2	WI-38	VERO	MCF-7
1	1	130.61	242.64	183.34	136.78
1	2	83.52	179.42	129.94	168.99
1	3	18.76	34.05	18.76	24.68
1	4	40.05	89.83	74.48	88.62
1	5	19.58	39.36	18.33	28
1	6	21.86	51.35	29.62	40.83
1	7	18.65	40.64	23.11	30.45
1	8	27.28	69.56	46.78	52.45
1	9	26.12	66.23	54.41	69.32
1	10	69.38	154.75	119.01	118.98
1	11	81.69	181.08	129.89	153.26
1	12	58.62	119.53	93.82	100.31
1	13	89.24	161.11	143.19	153.68
1	14	79.92	134.91	126.9	135.26
1	15	78.16	126.01	117.46	125.41
1	16	99.88	124.44	117.25	120.83
1	17	146.16	161.97	154.42	161.99
1	18	128.23	142.07	142.47	141.83
1	19	117.63	130.06	145.25	132.28
1	20	126.17	139.9	156.53	141.39
1	21	66.11	24.6	49.97	17.68
2	1	130.96	244.14	188.34	139.78
2	2	83.87	180.92	134.94	171.99
2	3	19.11	35.55	23.76	27.68
2	4	40.4	91.33	79.48	91.62
2	5	19.93	40.86	23.33	31
2	6	22.21	52.85	34.62	43.83
2	7	19	42.14	28.11	33.45
2	8	27.63	71.06	51.78	55.45
2	9	26.47	67.73	59.41	72.32
2	10	69.73	156.25	124.01	121.98
2	11	82.04	182.58	134.89	156.26
2	12	58.97	121.03	98.82	103.31
2	13	89.59	162.61	148.19	156.68
2	14	80.27	136.41	131.9	138.26
2	15	78.51	127.51	122.46	128.41
2	16	100.23	125.94	122.25	123.83
2	17	146.51	163.47	159.42	164.99
2	18	128.58	143.57	147.47	144.83
2	19	117.98	131.56	150.25	135.28
2	20	126.52	141.4	161.53	144.39
2	21	66.46	26.1	54.97	20.68
3	1	130.26	241.14	178.34	133.78
3	2	83.17	177.92	124.94	165.99
3	3	18.41	32.55	13.76	21.68
3	4	39.7	88.33	69.48	85.62
3	5	19.23	37.86	13.33	25
3	6	21.51	49.85	24.62	37.83
3	7	18.3	39.14	18.11	27.45
3	8	26.93	68.06	41.78	49.45
3	9	25.77	64.73	49.41	66.32
3	10	69.03	153.25	114.01	115.98
3	11	81.34	179.58	124.89	150.26
3	12	58.27	118.03	88.82	97.31
3	13	88.89	159.61	138.19	150.68
3	14	79.57	133.41	121.9	132.26
3	15	77.81	124.51	112.46	122.41
3	16	99.53	122.94	112.25	117.83
3	17	145.81	160.47	149.42	158.99
3	18	127.88	140.57	137.47	138.83
3	19	117.28	128.56	140.25	129.28
3	20	125.82	138.4	151.53	138.39
3	21	65.76	23.1	44.97	14.68

hydrogen bonding with one of its nucleotide bases. Furthermore, any compound with strong electron-withdrawing groups, like CO, CS, or CN, can interact electrostatically with DNA nucleobases and cause damage.

2.4. Statistical study

The provided data presents the susceptibility patterns of various cell lines including (*HepG2*, *WI-38*, *VERO*, and *MCF-7*) to different antibiotics. Each cell line was subjected to different antibiotics, and their susceptibility was measured across three different experimental conditions (labeled as 1, 2, and 3) (Tables 4–12). In conclusion, the statistical data obtained for all the newly synthesized compounds have a *P*-value less than 0.05 and this indicates that all the newly synthesized compounds are significant.

2.4.1. Analysis of variance

2.4.1.1. Variable: HepG2. Table 5.

2.4.1.2. Variable: WI-38. Table 7.

2.4.1.3. Variable: VERO. Table 9.

2.4.1.4. Variable: MCF-7. Table 11.

2.5. Molecular docking

Molecular docking studies are essential for predicting and understanding the interactions between synthesized compounds and biological targets, helping to assess their binding affinities, potential efficacy, and mechanism of action, which ultimately aids in optimizing the design of new therapeutic agents.

The selected derivatives (**5**, **7**, and **9**) were docked by captivating a selected PDBD: 2CLS protein, using the M.O.E program, and their fallouts were recognized (Table 13 and Fig. 2). Derivative **5** exhibited a binding energy of S = −7.8679 kcal/mol with RMSD = 1.7655. The interactions elaborated the 1st Pyrazole-ring with Gln 31 by π-H bond (4.57 Å), Benzene-ring with Lys 35 by π-H bond (3.72 Å), 1st Pyrazole-ring with Lys 35 by π-cation interaction (3.97 Å), 2nd Pyrazole-ring with Lys 126 by π-cation interaction (4.21 Å), and Pyrimidine-ring with Lys 126 by π-cation interaction (4.22 Å) Figure*). Meanwhile, derivative **7** revealed a good binding energy of S = −8.3013 kcal/mol with RMSD = 1.2311. The multiple interactions observed through C12 of 1st thiazole ring with Pro 39 by H-donor (3.34 Å), S14 of 2nd thiazole ring with Asp 128 by H-donor (3.13 Å), N19 of 1st triazole-ring with Thr 45 by H-acceptor (3.03 Å), N24 of 1st pyrimidine-ring with Thr 45 by H-acceptor (3.30 Å), C35 of 2nd pyrimidine ring with Phe 170 by H-π (4.86 Å), Triazole-ring N with Gln 23 by π-H (3.90 Å), 2nd Pyrimidine-ring with Gln 23 by π-H (4.64 Å), 1st thiazole ring with Tyr 42 by π-H (4.56 Å), 2nd Triazole-ring with Tyr 42 by π-H (4.04 Å), and 2nd Pyrimidine-ring with Tyr 42 by π-H (3.39 Å), indicate a stable and favorable binding conformation (Fig. 3). Moreover, derivative **9** revealed a proper binding energy of S = −7.8354 kcal/mol with RMSD = 1.2668. The various interactions detected through C12 of 1st thiazole ring with Asp 128 by H-donor (3.35 Å), S23 of 3rd

Table 5
Average of HepG2.

T	Total	
1	130.61	b
2	83.52	h
3	18.76	t
4	40.05	o
5	19.58	s
6	21.86	r
7	18.65	t
8	27.28	p
9	26.12	q
10	69.38	l
11	81.69	i
12	58.62	n
13	89.24	g
14	79.92	j
15	78.16	k
16	99.88	f
17	146.16	a
18	128.23	c
19	117.63	e
20	126.17	d
21	66.11	m
Grand Total	72.74380952	

Table 6
ANOVA Table.

EFFECT	SS	DF	MS	F	ProbF	
T	104376.4227	20	5218.821	42602.62	5.05E-84	**
Residual	5.145	42	0.1225			
Total	104381.5677	62	1683.574			
C.V. (%): 0.481140597802361						
S.E.M.: 0.20207259422045						
S.E.D.: 0.285773803330475						
LSD ($p < 0.05$): 0.576714883646069						
LSD ($p < 0.01$): 0.771036635673435						

Table 7
Average of WI-38.

T	Total	
1	242.64	a
2	179.42	b
3	34.05	o
4	89.83	j
5	39.36	n
6	51.35	m
7	40.64	n
8	69.56	k
9	66.23	l
10	154.75	d
11	181.08	b
12	119.53	i
13	161.11	c
14	134.91	f
15	126.01	h
16	124.44	h
17	161.97	c
18	142.07	e
19	130.06	g
20	139.9	e
21	24.6	p
Grand Total	114.9290476	

Table 8
ANOVA Table.

EFFECT	SS	DF	MS	F	ProbF	
T	204094.9289	20	10204.75	4535.443	1.34E-63	**
Residual	94.5	42	2.25			
Total	204189.4289	62	3293.378			
C.V. (%): 1.30515307580954						
S.E.M.: 0.866025403783638						
S.E.D.: 1.22474487139046						
LSD ($p < 0.05$): 2.47163521557381						
LSD ($p < 0.01$): 3.30444272424494						

thiazole-ring with Thr 45 by H-donor (3.60 Å), O14 of 1st pyrimidinone ring with Lys 126 by H-acceptor (3.21 Å), 4th thiazole ring with Gln 31 by π-H (3.94 Å), 3rd thiazole-ring with Tyr 42 by π-H (4.52 Å), 2nd pyrimidinone ring with Val 43 by π-H (3.49 Å), 4th thiazole ring with Val 43 by π-H (4.32 Å), and 2nd Triazole-ring with Lys 126 by π-cation (4.77 Å) (Fig. 4). Furthermore, **5-Flouro** discovered a weak binding energy of S = −4.5127 kcal/mol with higher RMSD = 0.5933. Their bindings were formed through N4 of pyrimidinedione ring with Asp 128 by H-donor (3.02 Å) and O6 of pyrimidinedione ring with Phe 170 by H-acceptor (3.12 Å) (Fig. 5).

3. Experiment

3.1. Chemistry

3.1.1. Synthesis of 1,1'-(1,4-phenylene)bis (2-bromoethan-1-one) (2)

This compound was prepared according to the previously reported work procedure [56].

Table 9
Average of VERO.

T	Total	
1	183.34	a
2	129.94	d
3	18.76	k
4	74.48	h
5	18.33	k
6	29.62	j
7	23.11	jk
8	46.78	i
9	54.41	i
10	119.01	ef
11	129.89	d
12	93.82	g
13	143.19	c
14	126.9	de
15	117.46	f
16	117.25	f
17	154.42	b
18	142.47	c
19	145.25	c
20	156.53	b
21	49.97	i
Grand Total	98.80619048	

Table 10
ANOVA Table.

EFFECT	SS	DF	MS	F	ProbF	
T	163515.8601	20	8175.793	327.0317	1.08E-39	**
Residual	1050	42	25			
Total	164565.8601	62	2654.288			
C.V. (%): 5.06041167653891						
S.E.M.: 2.88675134594837						
S.E.D.: 4.08248290463897						
LSD ($p < 0.05$): 8.23878405192101						
LSD ($p < 0.01$): 11.0148090808275						

Table 11
Average of MCF-7.

T	Total	
1	136.78	de
2	168.99	a
3	24.68	n
4	88.62	i
5	28	mn
6	40.83	l
7	30.45	m
8	52.45	k
9	69.32	j
10	118.98	g
11	153.26	c
12	100.31	h
13	153.68	c
14	135.26	e
15	125.41	f
16	120.83	fg
17	161.99	b
18	141.83	d
19	132.28	e
20	141.39	d
21	17.68	o
Grand Total	102.0485714	

Table 12
ANOVA Table.

EFFECT	SS	DF	MS	F	ProbF	
T	156230.9252	20	7811.546	867.9496	1.52E-48	**
Residual	378	42	9			
Total	156608.9252	62	2525.95			
C.V. (%): 2.93977657697868						
S.E.M.: 1.73205080756801						
S.E.D.: 2.44948974278195						
LSD ($p < 0.05$): 4.94327043114972						
LSD ($p < 0.01$): 6.60888544849267						

Table 13
Docking results of the synthesized derivatives with the higher score of MIC.

Der.	S (Kcal/mol)	RMSD	ligand bindings with the amino-acid residues	Types of Interactions	Distance (Å)
5	-7.8679	1.7655	1st Pyrazole-ring with Gln 31	Pi-H pi-H	4.57
			Benzene-ring with Lys 35	pi-cation	3.72
			1st Pyrazole-ring with Lys 35	pi-cation	3.97
			2nd Pyrazole-ring with Lys 126	pi-cation	4.21
			Pyrimidine-ring with Lys 126		4.22
7	-8.3013	1.2311	C 12 of 1st thiazole ring with Pro 39	H-donor	3.34
			S 14 of 2nd thiazole ring with Asp 128	H-donor	3.13
			N 19 of 1st triazole-ring with Thr 45	H-acceptor	3.03
			N 24 of 1st pyrimidine-ring Thr 45	H-acceptor	3.30
			C 35 of 2nd pyrimidine ring Phe 170	H-pi	4.86
			Triazole-ring N Gln 23	Pi-H	3.90
			2nd Pyrimidine-ring with Gln 23	Pi-H	4.64
			1st thiazole ring with Tyr 42	Pi-H	4.56
			2nd Triazole-ring with Tyr 42	Pi-H	4.04
			2nd Pyrimidine-ring with Tyr 42	Pi-H	3.39
9	-7.8354	1.2668	C 12of 1st thiazole ring with Asp 128	H-donor	3.35
			S 23 of 3rd thiazole-ring with Thr 45	H-donor	3.60
			O 14 of 1st pyrimidinone ring with LYS 126	H-acceptor pi-H pi-H	3.21
			4th thiazole ring with Gln 31	pi-H	3.94
			3rd thiazole-ring with Tyr 42	pi-H	4.52
			2nd pyrimidinone ring with Val 43	pi-cation	3.49
			4th thiazole ring with Val 43		4.32
			2nd Triazole-ring with Lys 126		4.77
5-Flouro	-4.5127	0.5933	N 4 of pyrimidinedione ring with Asp 128	H-donor	3.02
			O 6 of pyrimidinedione ring with Phe 170	H-acceptor	3.12

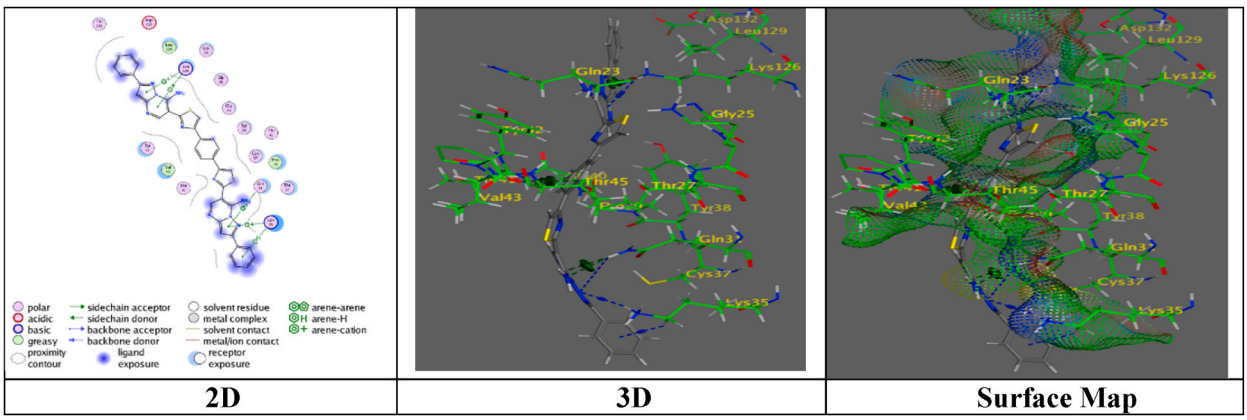


Fig. 2. Binding images between derivative **5** with PDB: 2CLS.

3.1.2. Synthesis of 2,2'-(1,4-phenylenebis (thiazole-4,2-diyl))diacetonitrile (3)

For 6 h, a solution of 2-cyanothioacetamide (0.22 g, 2 mmol) and bis(bromoacetyl) derivative **2** (0.3 g, 1 mmol) in dioxane (10 mL) was refluxed. Compound **3** was obtained by cooling, filtering, washing with cold ethanol, and recrystallizing the reaction mixture from glacial acetic acid.

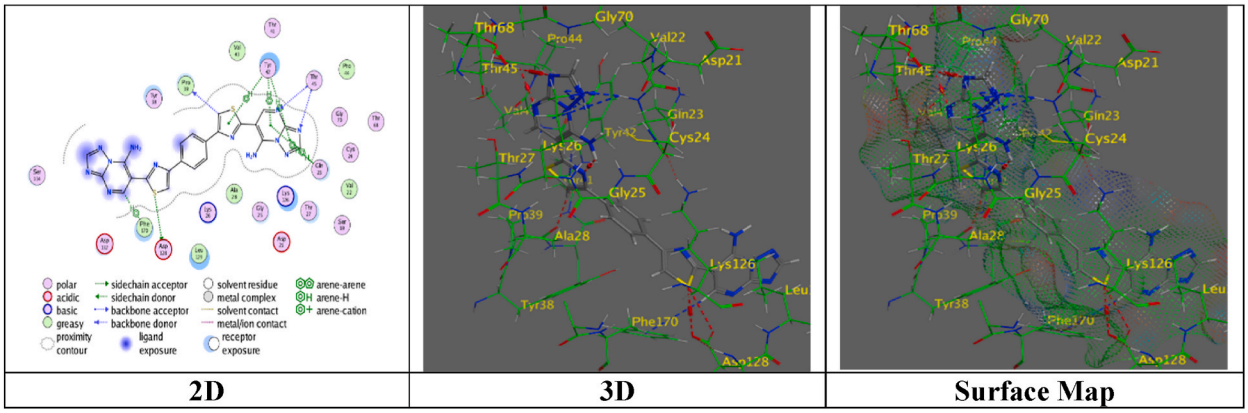


Fig. 3. Binding images between derivative **7** with PDB: 2CLS.

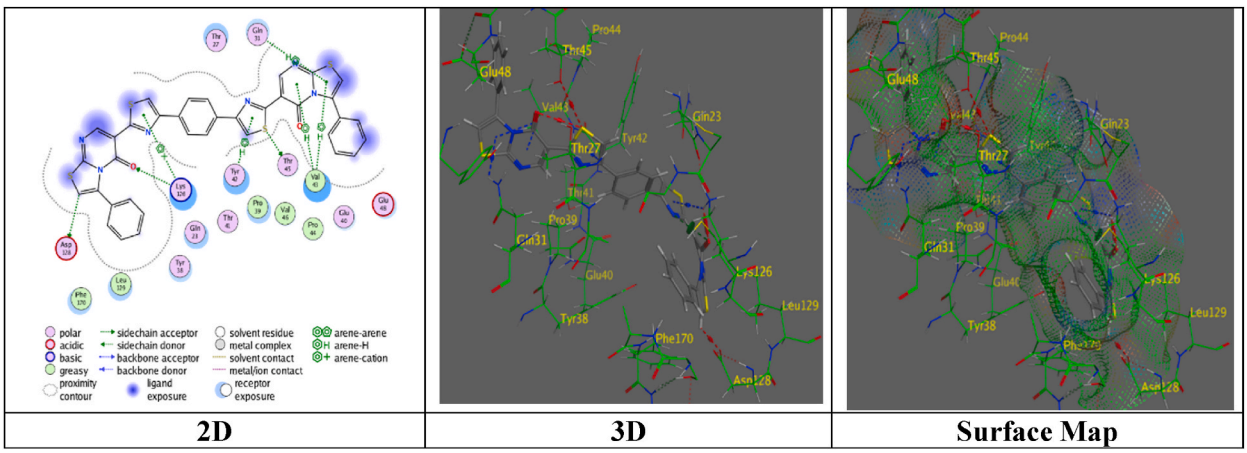


Fig. 4. Binding images between derivative **9** with PDB: 2CLS.

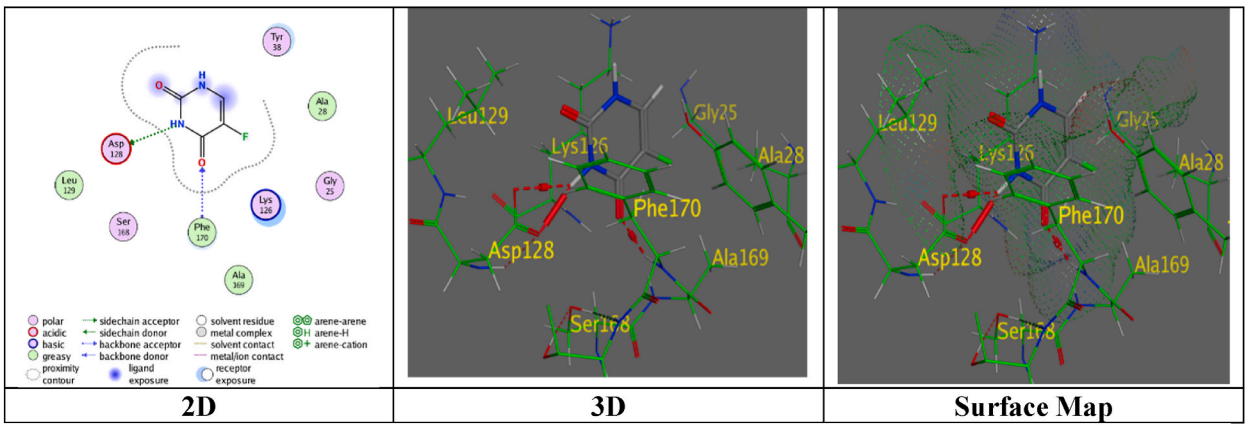


Fig. 5. Binding images between **5-Flouro** with PDB: 2CLS.

Yield, 73 %; m.p. 177–179 °C; IR (KBr): ν_{max}, cm^{-1}: 2220 (CN), 1615 (C=N); ^1H NMR (DMSO-d_6) δ ppm: 3.70 (s, 4H, $2CH_2$), 7.95 (s, 2H, thiazole-CH), 8.35 (s, 4H, Ar-H, symm. AB system); ^{13}C NMR (DMSO-d_6) δ ppm: 20.4 (2C), 108.0 (2C), 117.5 (2C), 128.2 (4C), 133.0 (2C), 153.7 (2C), 167.9 (2C). MS (*m/z*, %): 322 (M^+, 66). Anal. Calced for $C_{16}H_{10}N_4S_2$ (322.40): C, 59.61; H, 3.13; N, 17.38 %. Found: C, 59.58; H, 3.10; N, 17.26 %.

3.1.3. Synthesis of 2,2'-(1,4-phenylenebis (thiazole-4,2-diyl))bis (3-(dimethylamino)acrylonitrile) (4)

After refluxing for 3 h in dry toluene (20 mL) containing compound **3** (0.3 g, 1 mmol) and dimethylformamide-dimethylacetal (0.3 mL, 2.5 mmol), the mixture was allowed to cool at room temperature. Compound **4** was obtained by filtering off the orange precipitate result, washing it with petroleum ether, drying it thoroughly, and recrystallizing it from toluene.

Yield, 82 %; m.p. 228–230 °C; IR (KBr): ν_{max}, cm^{-1}: 2196 (CN), 1610 (C=N), 1580 (C=C); ^{1}H NMR (DMSO-d_6) δ ppm: 3.00 (s, 12H, 4CH_3), 6.80 (s, 2H, methine-CH), 8.15 (s, 2H, thiazole-CH), 8.50 (s, 4H, Ar-H, symm. AB system); ^{13}C NMR (DMSO-d_6) δ ppm: 44.1 (4C), 86.9 (2C), 110.6 (2C), 119.1 (2C), 128.5 (4C), 133.3 (2C), 152.1 (2C), 156.9 (2C), 165.1 (2C). MS (*m/z*, %): 432 (M^+, 62). Anal. Calced for $C_{22}H_{20}N_6S_2$ (432.56): C, 61.09; H, 4.66; N, 19.43 %. Found: C, 60.98; H, 4.59; N, 19.38 %.

3.1.4. Reaction of enaminonitrile 4 with heterocyclic amines

3.1.4.1. General procedure. Glacial acetic acid (6 mL) was mixed with enaminonitrile **4** (0.4 g, 1 mmol) in two folds of the corresponding heterocyclic amines. After being heated in an oil bath with reflux for 10–16 h, the mixture was vacuum-evaporated. Compounds **5**–**11** were produced by utilizing ethanol to triturate the residue, then filtering, collecting, and completely drying the solid product before recrystallizing it in the appropriate solvent.

3.1.5. 6,6'-(1,4-Phenylenebis (thiazole-4,2-diyl))bis (2-phenylpyrazolo [1,5-a]pyrimidin-7-amine) (5)

Yield, 76 %; m.p. 266–268 °C; IR (KBr): ν_{max}, cm^{-1}: 3350 (NH_2), 1620 (C=N); ^{1}H NMR (DMSO-d_6) δ ppm: 6.71 (s, 2H, pyrazole-CH), 6.90 (s, 4H, 2NH_2), 7.48–7.70 (m, 10H, Ar-H), 7.78 (s, 2H, pyrimidine-CH), 8.44 (s, 4H, Ar-H, symm. AB system), 8.79 (s, 2H, thiazole-CH); ^{13}C NMR (DMSO-d_6) δ ppm: 92.9 (2C), 110.0 (2C), 116.6 (2C), 126.0 (4C), 127.4 (4C), 128.8 (2C), 130.1 (4C), 134.0 (4C), 149.7 (2C), 150.4 (2C), 152.0 (2C), 154.5 (2C), 157.5 (2C), 166.0 (2C). MS (*m/z*, %): 660 (M^+, 59), 451 (55), 369 (58), 292 (100), 209 (50), 193 (42), 132 (49), 116 (34), 88 (42), 83 (33), 77 (88), 76 (33). Anal. Calced for $C_{36}H_{24}N_{10}S_2$ (660.78): C, 65.44; H, 3.66; N, 21.20 %. Found: C, 65.38; H, 3.60; N, 21.15 %.

3.1.6. 6,6'-(1,4-Phenylenebis (thiazole-4,2-diyl))bis (tetrazolo [1,5-a]pyrimidin-7(3H)-one) (6)

Yield, 71 %; m.p. 248–250 °C; IR (KBr): ν_{max}, cm^{-1}: 3220 (NH), 1680 (amidic CO), 1610 (C=N); ^{1}H NMR (DMSO-d_6) δ ppm: 8.28 (s, 2H, thiazole-CH), 8.60 (s, 4H, Ar-H, symm. AB system), 8.99 (s, 2H, pyrimidine-CH), 9.50 (s, 2H, 2NH); ^{13}C NMR (DMSO-d_6) δ ppm: 110.8 (2C), 128.1 (4C), 133.7 (2C), 134.8 (2C), 152.8 (4C), 154.0 (2C), 166.1 (4C). MS (*m/z*, %): 514 (M^+, 55), 513 (33), 512 (14), 397 (48), 295 (40), 219 (61), 136 (100), 108 (47), 83 (32), 80 (71), 76 (84). Anal. Calced for $C_{20}H_{10}N_{12}O_2S_2$ (514.50): C, 46.69; H, 1.96; N, 32.67 %. Found: C, 46.60; H, 1.89; N, 32.59 %.

3.1.7. 6,6'-(1,4-Phenylenebis (thiazole-4,2-diyl))bis ([1,2,4]triazolo [1,5-a]pyrimidin-7-amine) (7)

Yield, 77 %; m.p. 221–223 °C; IR (KBr): ν_{max}, cm^{-1}: 3380 (NH_2), 1615 (C=N); ^{1}H NMR (DMSO-d_6) δ ppm: 6.76 (s, 4H, 2NH_2), 7.89 (s, 2H, pyrimidine-CH), 8.31 (s, 4H, Ar-H, symm. AB system), 8.69 (s, 2H, thiazole-CH), 8.88 (s, 2H, triazole-CH); ^{13}C NMR (DMSO-d_6) δ ppm: 109.9 (2C), 116.4 (2C), 128.9 (4C), 133.9 (2C), 150.2 (2C), 152.0 (2C), 154.2 (4C), 157.1 (2C), 167.8 (2C). MS (*m/z*, %): 510 (M^+, 57), 376 (50), 293 (56), 217 (41), 201 (38), 134 (60), 118 (100), 91 (45), 83 (65), 76 (84), 64 (43). Anal. Calced for $C_{22}H_{14}N_{12}S_2$ (510.56): C, 51.76; H, 2.76; N, 32.92 %. Found: C, 51.71; H, 2.69; N, 32.88 %.

3.1.8. 3,3'-(1,4-Phenylenebis (thiazole-4,2-diyl))bis (4H-benzo [4,5]thiazolo [3,2-a]pyrimidin-4-one) (8)

Yield, 71 %; m.p. 282–284 °C; IR (KBr): ν_{max}, cm^{-1}: 1685 (amidic CO), 1615 (C=N); ^{1}H NMR (DMSO-d_6) δ ppm: 7.50–7.66 (m, 8H, Ar-H), 8.11 (s, 2H, thiazole-CH), 8.25 (s, 4H, Ar-H, symm. AB system), 8.40 (s, 2H, pyrimidine-CH); ^{13}C NMR (DMSO-d_6) δ ppm: 110.8 (2C), 122.7 (2C), 126.8 (4C), 128.0 (2C), 129.8 (4C), 130.9 (2C), 133.8 (2C), 136.0 (2C), 136.9 (2C), 151.4 (2C), 152.9 (2C), 157.9 (2C), 164.0 (2C), 167.8 (2C). MS (*m/z*, %): 644 (M^+, 58), 443 (40), 360 (61), 284 (67), 201 (41), 173 (100), 148 (59), 122 (45), 108 (61), 83 (35), 76 (60). Anal. Calced for $C_{32}H_{16}N_6O_2S_4$ (644.76): C, 59.61; H, 2.50; N, 13.03 %. Found: C, 59.56; H, 2.45; N, 12.96 %.

3.1.9. 6,6'-(1,4-Phenylenebis (thiazole-4,2-diyl))bis (3-phenyl-5H-thiazolo [3,2-a]pyrimidin-5-one) (9)

Yield, 78 %; m.p. 248–250 °C; IR (KBr): ν_{max}, cm^{-1}: 1675 (amidic CO), 1612 (C=N); ^{1}H NMR (DMSO-d_6) δ ppm: 6.30 (s, 2H, fused thiazole-CH), 7.60–7.76 (m, 10H, Ar-H), 8.20 (s, 2H, thiazole-CH), 8.33 (s, 4H, Ar-H, symm. AB system), 8.49 (s, 2H, pyrimidine-CH); ^{13}C NMR (DMSO-d_6) δ ppm: 111.4 (2C), 111.9 (2C), 127.9 (2C), 128.7 (4C), 129.8 (4C), 130.8 (4C), 134.2 (2C), 136.0 (4C), 147.6 (2C), 152.4 (2C), 156.0 (2C), 159.7 (2C), 163.0 (2C), 164.7 (2C). MS (*m/z*, %): 696 (M^+, 58), 619 (54), 469 (61), 386 (41), 310 (48), 233 (35), 150 (100), 122 (66), 99 (40), 83 (62), 77 (82), 76 (56). Anal. Calced for $C_{36}H_{20}N_6O_2S_4$ (696.84): C, 62.05; H, 2.89; N, 12.06 %. Found: C, 61.96; H, 2.80; N, 11.97 %.

3.1.10. 3,3'-(1,4-Phenylenebis (thiazole-4,2-diyl))bis (8-methyl-4H-pyrido [1,2-a]pyrimidin-4-one) (10)

Yield, 81 %; m.p. 276–278 °C; IR (KBr): ν_{max}, cm^{-1}: 1681 (amidic CO), 1615 (C=N), 1580 (C=C); ^{1}H NMR (DMSO-d_6) δ ppm: 2.25 (s, 6H, 2CH_3), 7.09 (d, 2H, pyridine-CH), 7.20 (s, 2H, pyridine-CH), 8.29 (s, 2H, thiazole-CH), 8.40 (s, 4H, Ar-H, symm. AB system), 8.82 (d, 2H, pyridine-CH), 9.06 (s, 2H, pyrimidine-CH); ^{13}C NMR (DMSO-d_6) δ ppm: 18.4 (2C), 110.0 (2C), 115.4 (2C), 123.3 (2C), 126.1 (2C), 127.5 (4C), 134.0 (2C), 134.8 (2C), 145.5 (2C), 150.0 (2C), 151.8 (2C), 153.4 (2C), 163.4 (2C), 164.7 (2C). MS (*m/z*, %): 560 (M^+, 50), 554 (76), 401 (54), 318 (46), 242 (53), 159 (100), 144 (43), 116 (73), 91 (60), 83 (74), 76 (88). Anal. Calced for $C_{30}H_{20}N_6O_2S_2$ (560.65): C, 64.27; H, 3.60; N, 14.99 %. Found: C, 64.17; H, 3.53; N, 14.89 %.

3.1.11. 3,3'-(1,4-Phenylenebis (thiazole-4,2-diyl))bis (benzo [4,5]imidazo [1,2-a]pyrimidin-4-amine) (11)

Yield, 70 %; m.p. 281–283 °C; IR (KBr): ν_{max}, cm^{-1}: 3450–3320 (NH_2), 1610 (C=N); ^1H NMR (DMSO-d_6) δ ppm: 6.65 (s, 4H, $2NH_2$), 7.20–7.31 (m, 6H, Ar-H), 7.80 (s, 2H, pyrimidine-CH), 8.35 (s, 4H, Ar-H, symm. AB system), 8.67 (d, 2H, Ar-H), 8.81 (s, 2H, thiazole-CH); ^{13}C NMR (DMSO-d_6) δ ppm: 110.6 (2C), 112.0 (2C), 116.7 (2C), 120.0 (2C), 123.6 (4C), 128.1 (4C), 133.4 (2C), 136.5 (2C), 143.9 (2C), 149.8 (2C), 150.2 (2C), 152.0 (2C), 154.6 (2C), 168.4 (2C). MS (*m/z*, %): 608 (M^+, 54), 425 (48), 342 (56), 266 (58), 183 (48), 167 (100), 141 (50), 116 (32), 83 (74), 76 (50). Anal. Calced for $C_{32}H_{20}N_{10}S_2$ (608.70): C, 63.14; H, 3.31; N, 23.01 %. Found: C, 63.07; H, 3.29; N, 22.95 %.

3.1.12. Reaction of enaminonitrile 4 with N-nucleophiles

3.1.12.1. General procedure. Anhydrous potassium carbonate (0.28 g, 2 mmol) (a catalytic amount of piperidine 3 drops was used in case of thiourea), hydrazine hydrate (80 %, 0.1 mL, 2 mmol), guanidine nitrate (0.24 g, 2 mmol), and enaminonitrile **4** (0.4 g, 1 mmol) were added to a mixture of ethanol and dimethylformamide (1:1) (30 mL) and heated under reflux for 10 h before being allowed to cool at room temperature. A few drops of diluted HCl were added to the reaction mixture (until $pH = 7$) after it had been triturated with 50 mL of cold water. After the precipitated solid product was filtered, completely dried, and crystallized from a solution of ethanol and dimethylformamide (1:1), compounds **12**–**15** were extracted.

3.1.13. 4,4'-(1,4-Phenylenebis (thiazole-4,2-diyl))bis (isoxazol-5-amine) (12)

Yield, 76 %; m.p. 177–179 °C; IR (KBr): ν_{max}, cm^{-1}: 3400–3350 (NH_2), 1620 (C=N); ^1H NMR (DMSO-d_6) δ ppm: 6.35 (s, 4H, $2NH_2$), 8.07 (s, 2H, isoxazole-CH), 8.40 (s, 4H, Ar-H, symm. AB system), 8.77 (s, 2H, thiazole-CH); ^{13}C NMR (DMSO-d_6) δ ppm: 100.1 (2C), 111.0 (2C), 129.9 (4C), 135.0 (2C), 151.7 (2C), 153.0 (2C), 155.8 (2C), 158.1 (2C). MS (*m/z*, %): 408 (M^+, 56), 392 (67), 376 (62), 226 (45), 150 (100), 83 (74), 76 (64), 67 (46). Anal. Calced for $C_{18}H_{12}N_6O_2S_2$ (408.45): C, 52.93; H, 2.96; N, 20.58 %. Found: C, 52.88; H, 2.86; N, 20.50 %.

3.1.14. 4,4'-(1,4-Phenylenebis (thiazole-4,2-diyl))bis (1H-pyrazol-5-amine) (13)

Yield, 79 %; m.p. 153–155 °C; IR (KBr): ν_{max}, cm^{-1}: 3410 (NH_2), 3280 (NH), 1610 (C=N); ^1H NMR (DMSO-d_6) δ ppm: 5.30 (s, 4H, $2NH_2$), 7.98 (s, 2H, pyrazole-CH), 8.31 (s, 4H, Ar-H, symm. AB system), 8.69 (s, 2H, thiazole-CH), 12.70 (s, 2H, 2NH); ^{13}C NMR (DMSO-d_6) δ ppm: 91.9 (2C), 110.2 (2C), 129.5 (4C), 132.0 (2C), 133.7 (2C), 134.3 (2C), 152.4 (2C), 155.0 (2C). MS (*m/z*, %): 406 (M^+, 60), 390 (62), 374 (51), 241 (56), 225 (49), 165 (70), 156 (52), 149 (100), 83 (66), 82 (60), 76 (40), 66 (42). Anal. Calced for $C_{18}H_{14}N_8S_2$ (406.49): C, 53.19; H, 3.47; N, 27.57 %. Found: C, 53.10; H, 3.38; N, 27.49 %.

3.1.15. 5,5'-(1,4-Phenylenebis (thiazole-4,2-diyl))bis (pyrimidine-2,4-diamine) (14)

Yield, 80 %; m.p. 189–191 °C; IR (KBr): ν_{max}, cm^{-1}: 3410–3250 (NH_2), 1615 (C=N); ^1H NMR (DMSO-d_6) δ ppm: 6.46 (s, 4H, $2NH_2$), 7.10 (s, 4H, $2NH_2$), 8.16 (s, 2H, pyrimidine-CH), 8.48 (s, 4H, Ar-H, symm. AB system), 8.82 (s, 2H, thiazole-CH); ^{13}C NMR (DMSO-d_6) δ ppm: 106.1 (2C), 110.0 (2C), 128.9 (4C), 133.0 (2C), 150.9 (2C), 152.0 (2C), 154.6 (2C), 157.8 (2C), 162.8 (2C). MS (*m/z*, %): 460 (M^+, 55), 444 (60), 428 (50), 412 (66), 396 (57), 268 (71), 236 (69), 192 (36), 160 (100), 109 (69), 93 (62), 83 (84), 77 (74), 76 (56), 51 (44). Anal. Calced for $C_{20}H_{16}N_{10}S_2$ (460.54): C, 52.16; H, 3.50; N, 30.41 %. Found: C, 52.10; H, 3.46; N, 30.38 %.

3.1.16. 5,5'-(1,4-Phenylenebis (thiazole-4,2-diyl))bis (2-thioxo-2,3-dihydropyrimidin-4(1H)-one) (15)

Yield, 72 %; m.p. 173–175 °C; IR (KBr): ν_{max}, cm^{-1}: 3395–3162 (NH), 1680 (amidic CO), 1625 (C=N), 1279 (C=S); ^1H NMR (DMSO-d_6) δ ppm: 7.15 (s, 2H, pyrimidine-CH), 7.50 (s, 2H, 2NH), 8.25 (s, 2H, thiazole-CH), 8.59 (s, 4H, Ar-H, symm. AB system), 12.60 (s, 2H, 2NH); ^{13}C NMR (DMSO-d_6) δ ppm: 109.6 (2C), 116.0 (2C), 128.3 (4C), 133.6 (2C), 151.5 (2C), 154.0 (2C), 164.0 (2C), 165.5 (2C), 175.7 (2C). MS (*m/z*, %): 496 (M^+, 56), 494 (63), 492 (51), 369 (42), 286 (55), 210 (100), 159 (62), 127 (55), 99 (50), 83 (75), 76 (47), 74 (61). Anal. Calced for $C_{20}H_{12}N_6O_2S_4$ (496.60): C, 48.37; H, 2.44; N, 16.92 %. Found: C, 48.29; H, 2.39; N, 16.87 %.

3.1.17. Reaction of enaminonitrile 4 with active methylene group incorporated heterocyclic compounds

3.1.17.1. General procedure. Enaminonitrile **4** (0.4 g, 1 mmol) in glacial acetic acid (6 mL) was dissolved, and two folds of the active methylene integrated heterocyclic compounds were added. The mixture was heated for six to 10 h under reflux in an oil bath before being evaporated in a vacuum. Compounds **18**–**20** were produced by utilizing ethanol to triturate the residue, sifting and collecting the solid product, then completely drying and recrystallizing it in the appropriate solvent.

3.1.18. 6,6'-(1,4-Phenylenebis (thiazole-4,2-diyl))bis (2H-pyrano [2,3-d]pyrimidine-2,4,7(1H,3H)-trione) (18)

Barbituric acid (0.26 g, 2 mmol) was heated for 10 h under reflux to synthesis this compound, which was then recrystallized from ethanol and dimethylformamide (1:1).

Yield, 77 %; m.p. 215–217 °C; IR (KBr): ν_{max}, cm^{-1}: 3260 (NH), 1720 (pyran CO), 1685 (amidic CO), 1610 (C=N), 1585 (C=C); ^1H NMR (DMSO-d_6) δ ppm: 8.00 (s, 2H, pyran-CH), 8.37 (s, 2H, thiazole-CH), 8.69 (s, 4H, Ar-H, symm. AB system), 10.51 (s, 2H, 2NH), 10.91 (s, 2H, 2NH); ^{13}C NMR (DMSO-d_6) δ ppm: 87.3 (2C), 111.2 (2C), 130.0 (4C), 133.9 (2C), 135.1 (2C), 141.9 (2C), 150.1 (2C), 152.8 (2C), 153.9 (4C), 162.9 (2C), 165.1 (2C). MS (*m/z*, %): 600 (M^+, 61), 421 (38), 338 (43), 263 (32), 242 (43), 179 (39), 159 (50), 135 (46), 110 (68), 83 (100), 76 (53), 58 (52), 52 (46). Anal. Calced for $C_{26}H_{12}N_6O_8S_2$ (600.54): C, 52.00; H, 2.01; N, 13.99 %. Found:

C, 51.91; H, 1.96; N, 13.90 %.

3.1.19. 5,5'-(1,4-Phenylenebis (thiazole-4,2-diyl))bis (3-phenyl-6H-pyrano [3,2-d]isoxazol-6-one) (19)

This compound was synthesized by heating 3-phenylisoxazole-5-one (0.32 g, 2 mmol) for 6 h under reflux, and then recrystallizing it using a 1:1 mixture of ethanol and dimethylformamide.

Yield, 73 %; m.p. 198–200 °C; IR (KBr): ν_{max}, cm^{-1}: 1730 (pyran CO), 1615 (C=N), 1580 (C=C); ^{1}H NMR (DMSO-d_6) δ ppm: 7.31 (s, 2H, pyran-CH), 7.60–8.00 (m, 10H, Ar-H), 8.10 (s, 2H, thiazole-CH), 8.18 (s, 4H, Ar-H, symm. AB system); ^{13}C NMR (DMSO-d_6) δ ppm: 99.9 (2C), 111.3 (2C), 127.1 (4C), 128.0 (4C), 128.9 (4C), 130.0 (4C), 133.8 (2C), 135.1 (2C), 146.9 (2C), 151.8 (2C), 158.5 (2C), 160.8 (2C), 162.1 (2C), 166.0 (2C). MS (*m/z*, %): 666 (M^+, 66), 589 (50), 512 (56), 454 (47), 371 (62), 295 (57), 242 (67), 218 (60), 212 (50), 135 (70), 91 (72), 84 (100), 77 (69), 78 (39), 66 (36). Anal. Calced for $C_{36}H_{18}N_4O_6S_2$ (666.68): C, 64.86; H, 2.72; N, 8.40 %. Found: C, 64.80; H, 2.68; N, 8.39 %.

3.1.20. 5,5'-(1,4-Phenylenebis (thiazole-4,2-diyl))bis (3-methyl-1-phenylpyrano [2,3-c]pyrazol-6(1H)-one) (20)

This compound was synthesized by heating (0.34 g, 2 mmol) of 3-methyl-1-phenyl-1Hpyrazole-5(4H)-one for 10 h under reflux, and then recrystallized from ethanol and dimethylformamide (1:1).

Yield, 76 %; m.p. 182–184 °C; IR (KBr): ν_{max}, cm^{-1}: 1720 (pyran CO), 1615 (C=N), 1582 (C=C); ^{1}H NMR (DMSO-d_6) δ ppm: 2.30 (s, 6H, $2CH_3$), 7.49 (s, 2H, pyran-CH), 7.70–7.86 (m, 10H, Ar-H), 8.02 (s, 2H, thiazole-CH), 8.39 (s, 4H, Ar-H, symm. AB system); ^{13}C NMR (DMSO-d_6) δ ppm: 14.0 (2C), 110.5 (2C), 114.6 (2C), 121.9 (4C), 126.4 (2C), 128.2 (4C), 129.8 (4C), 133.1 (2C), 134.7 (2C), 138.0 (2C), 146.1 (2C), 148.3 (2C), 155.9 (4C), 161.9 (2C), 165.6 (2C). MS (*m/z*, %): 692 (M^+, 63), 467 (56), 384 (61), 308 (39), 293 (62), 225 (100), 216 (45), 210 (67), 133 (48), 89 (65), 83 (82), 77 (78), 76 (44), 64 (61), 44 (54). Anal. Calced for $C_{38}H_{24}N_6O_4S_2$ (692.77): C, 65.88; H, 3.49; N, 12.13 %. Found: C, 65.80; H, 3.41; N, 12.05 %.

3.1.21. Reaction of enaminonitrile 4 with C-nucleophiles

3.1.21.1. General procedure. Two folds of the suitable *C*-nucleophiles were added to a solution of enaminonitrile **4** (0.4 g, 1 mmol) in glacial acetic acid (6 mL). The mixture was heated in an oil bath under reflux for 10–12 h, and it was then evaporated in vacuo. Compounds **21**–**24** were obtained by triturating the residue with ethanol, collecting the solid product by filtration, thoroughly drying it, and recrystallizing it from the suitable solvent.

3.1.22. 3,3'-(1,4-Phenylenebis (thiazole-4,2-diyl))bis (5-acetyl-6-methyl-2H-pyran-2-one) (21)

This compound was synthesized by heating acetylacetone (0.26 g, 2 mmol) for 10 h under reflux, and then recrystallized from a 1:1 mixture of ethanol and dimethylformamide.

Yield, 74 %; m.p. 210–212 °C; IR (KBr): ν_{max}, cm^{-1}: 1725 (CO), 1710 (pyran CO), 1610 (C=N), 1590 (C=C); ^{1}H NMR (DMSO-d_6) δ ppm: 2.29 (s, 6H, $2CO\text{-}CH_3$), 2.39 (s, 6H, $2CH_3$), 7.77 (s, 2H, pyran-CH), 8.10 (s, 2H, thiazole-CH), 8.40 (s, 4H, Ar-H, symm. AB system); ^{13}C NMR (DMSO-d_6) δ ppm: 20.0 (2C), 30.7 (2C), 110.8 (2C), 117.8 (2C), 127.9 (4C), 133.4 (2C), 134.1 (2C), 141.8 (2C), 152.2 (2C), 153.6 (2C), 164.7 (4C), 196.5 (2C). MS (*m/z*, %): 544 (M^+, 51), 529 (42), 514 (64), 471 (44), 428 (41), 394 (51), 310 (50), 242 (70), 234 (100), 159 (48), 152 (44), 136 (60), 121 (56), 108 (54), 94 (50), 83 (70), 76 (42), 49 (46). Anal. Calced for $C_{28}H_{20}N_2O_6S_2$ (544.60): C, 61.75; H, 3.70; N, 5.14 %. Found: C, 61.69; H, 3.66; N, 5.01 %.

3,3'-(1,4-Phenylenebis (thiazole-4,2-diyl))bis (7,7-dimethyl-7,8-dihydro-2H-chromene-2,5(6H)-dione) (22):

This compound was synthesized by heating dimedone (0.28 g, 2 mmol) for 10 h under reflux, and then recrystallizing it using a 1:1 mixture of ethanol and dimethylformamide.

Yield, 69 %; m.p. 264–266 °C; IR (KBr): ν_{max}, cm^{-1}: 1715 (pyran CO), 1690 (cyclic CO), 1620 (C=N), 1585 (C=C); ^{1}H NMR (DMSO-d_6) δ ppm: 1.26 (s, 12H, $4CH_3$), 1.90 (s, 4H, chromene-C_8-H), 2.31 (s, 4H, chromene-C_6-H), 7.80 (s, 2H, chromene-C_4-H), 8.28 (s, 2H, thiazole-CH), 8.44 (s, 4H, Ar-H, symm. AB system); ^{13}C NMR (DMSO-d_6) δ ppm: 26.9 (4C), 32.7 (2C), 43.7 (4C), 109.1 (2C), 115.9 (2C), 128.1 (4C), 133.8 (2C), 134.2 (2C), 142.9 (2C), 151.7 (2C), 152.7 (2C), 153.9 (2C), 165.4 (2C), 194.1 (2C). MS (*m/z*, %): 624 (M^+, 50), 594 (46), 564 (52), 403 (55), 350 (67), 274 (50), 242 (68), 191 (65), 161 (60), 159 (45), 147 (24), 122 (66), 94 (48), 83 (100), 76 (74), 70 (41), 44 (64). Anal. Calced for $C_{34}H_{28}N_2O_6S_2$ (624.73): C, 65.37; H, 4.52; N, 4.48 %. Found: C, 65.27; H, 4.48; N, 4.39 %.

3.1.23. 2,2'-(1,4-Phenylenebis (thiazole-4,2-diyl))bis (1-oxo-1H-benzo [4,5]thiazolo [3,2-a]pyridine-4-carbonitrile) (23)

This compound was synthesized by heating benzothiazole-2-acetonitrile (0.3 g, 2 mmol) for 12 h under reflux, and then recrystallizing it using a 1:1 mixture of ethanol and dimethylformamide.

Yield, 66 %; m.p. 286–288 °C; IR (KBr): ν_{max}, cm^{-1}: 2219 (CN), 1685 (amidic CO), 1620 (C=N), 1580 (C=C); ^{1}H NMR (DMSO-d_6) δ ppm: 7.70 (s, 2H, pyridine-CH), 7.87–8.05 (m, 8H, Ar-H), 8.49 (s, 2H, thiazole-CH), 8.79 (s, 4H, Ar-H, symm. AB system); ^{13}C NMR (DMSO-d_6) δ ppm: 73.5 (2C), 111.7 (2C), 115.8 (2C), 122.2 (2C), 124.0 (2C), 125.6 (2C), 126.6 (2C), 128.5 (4C), 130.0 (2C), 134.8 (2C), 139.8 (2C), 142.5 (4C), 153.8 (2C), 159.0 (2C), 163.9 (2C), 165.7 (2C). MS (*m/z*, %): 692 (M^+, 53), 467 (39), 384 (49), 308 (69), 242 (53), 225 (100), 199 (60), 171 (64), 159 (65), 147 (59), 83 (70), 76 (44), 70 (65). Anal. Calced for $C_{36}H_{16}N_6O_2S_4$ (692.80): C, 62.41; H, 2.33; N, 12.13 %. Found: C, 62.39; H, 2.27; N, 12.05 %.

3.1.24. 2,2'-(1,4-Phenylenebis (thiazole-4,2-diyl))bis (1-oxo-1,5-dihydrobenzo [4,5]imidazo [1,2-a]pyridine-4-carbonitrile) (24)

This compound was synthesized by heating benzimidazole-2-acetonitrile (0.3 g, 2 mmol) for 12 h under reflux, and then recrystallizing it using a 1:1 mixture of ethanol and dimethylformamide.

Yield, 65 %; m.p. 222–224 °C; IR (KBr): ν_{max}, cm^{-1}: 3220 (NH), 2220 (CN), 1675 (amidic CO), 1615 (C=N), 1590 (C=C); ^{1}H NMR (DMSO-d_6) δ ppm: 6.89–7.05 (m, 6H, Ar-H), 7.68 (s, 2H, pyridine-CH), 7.84 (d, 2H, Ar-H), 8.28 (s, 2H, thiazole-CH), 8.61 (s, 4H, Ar-H, symm. AB system), 10.88 (s, 2H, 2NH); ^{13}C NMR (DMSO-d_6) δ ppm: 59.4 (2C), 110.2 (2C), 114.0 (2C), 115.9 (2C), 121.1 (2C), 122.6 (2C), 124.0 (2C), 125.3 (2C), 127.4 (4C), 133.7 (2C), 138.0 (2C), 141.2 (4C), 152.1 (2C), 157.7 (2C), 162.1 (2C), 164.0 (2C). MS (*m/z*, %): 658 (M^+, 61), 450 (46), 367 (57), 291 (61), 242 (70), 208 (100), 182 (57), 159 (44), 154 (37), 129 (55), 83 (73), 76 (61), 53 (46). Anal. Calced for $C_{36}H_{18}N_8O_2S_2$ (658.71): C, 65.64; H, 2.75; N, 17.01 %. Found: C, 65.58; H, 2.68; N, 16.96 %.

3.2. Biological evaluation

3.2.1. Anti-Herpes Simplex-1 Virus (HSV-1) In vitro [57]:

Samples were generated by serially dilution and plating in microtiter trays after being dissolved in DMSO and aliquots diluted into sterile culture medium. Confluent monolayer cultures of Vero cells in microtiter trays were inverted. After shaking out the medium, it was replaced with three separate serial dilutions of sterile extracts in 100 mL of medium, then a tittered virus in 100 mL of medium with 10 % (v/v) calf serum in each cell. The final row of cells in each tray was set aside for controls, meaning that neither a chemical nor a virus was applied. For 6 h, the trays were cultivated and incubated at 37 °C in an environment of 5 % CO_2. Turned upside down, the trays become a pad of paper towels. After carefully rinsing the remaining cells with a medium, they were fixed for 20 min with 3.7 % (v/v) formaldehyde in saline. After being cleaned with water, the tiny cells were inspected. Relatively confluent, unmodified monolayers of labeled Vero cells treated with HSV-1 are indicative of antiviral action. The concentration that resulted in roughly a 50 % loss of the monolayer around the HSV-1-caused plaques was used to evaluate cytotoxicity (Table 1).

3.2.2. Anti-Human Immunodeficiency Virus-1 (HIV-1) In vitro [57]:

To prepare compounds for the test, they were first dissolved in DMSO, diluted 1:100 in a cell culture medium, and then prepared for serial dilution, which was then put onto microtiter trays. Following the addition of T4 lymphocytes (CEM cell line) and HIV-1, which was added after a brief interval of at least 1 min, each of the investigated drugs was diluted to a final concentration of 1:200. For six days, cultures were incubated at 37 °C in an environment 5 % CO2. All cells were treated with tetrazolium salt XTT, and the cultures were allowed to incubate so that the virus-infected cells could develop the formazan color. A quantitative formazan production was obtained by spectrophotometric analysis of individual cells, which were also examined under a microscope to identify any alive cells. The results were compared to positive controls, cells treated with zidovudine (AZT) as a control, and the proportion of T4 cells that were protected from the HIV-1 cytopathic effect was used to calculate the activity (Table 2).

3.2.3. Antitumor activity [58]:

RPMI-1640 medium was used to dilute the stock samples to the necessary quantities, which ranged from 1 to 50 μg/mL. Dimethyl sulfoxide (DMSO) at the end concentration in each sample was kept below 1 % v/v. African green monkey kidney (VERO), human breast adenocarcinoma (MCF-7) cell lines, lung fibroblast (WI-38), hepatoma (HepG2), and lung fibroblast (WI-38) growth were used to investigate the compounds' cytotoxic activities. The cell's vitality was visually assessed. 5-fluorouracil, a common anticancer medication, was utilized as a point of comparison.

Briefly said, cells were batch-grown for 10 days before being seeded in 96-well microtiter plastic plates at a density of 10×10^3 cells/well in a new complete growth medium. The experiment was conducted using a water-jacketed carbon dioxide incubator (Shedon.TC2323.Cornelius, OR, USA) at 37 °C for 24 h under 5 % CO_2. After adding the medium (serum-free), the cells were cultured either in the absence of medium (negative control) or with varying doses ranging from 1 to 50 μg/mL. In 96-well flat bottom microplates with 5 % CO_2, 1 % L-glutamin, 104 μg/mL potassium penicillin, 104 μg/mL streptomycin sulfate, and 25 μg/mL amphotericin B, as well as RPMI-1640 medium were used to suspend the cells. Following a 96-h incubation period, the medium was once more aspirated, the trays were turned over onto a pad of paper towels, and the surviving cells were gently washed with the medium before being fixed for at least 20 min with 3.7 % (v/v) formaldehyde in saline. After being cleaned with water, the fixed cells were inspected. Confluent, largely unchanged monolayers of dyed cells treated with chemicals were found to exhibit cytotoxic action.

3.3. Statistical analysis

All results were analyzed using descriptive statistical techniques such as mean and standard deviation. One way ANOVA was employed to test the significance and was considered statistically significant.

3.4. Molecular docking study

The M.O.E. 2019.0102 molecular docking tool correctly simulates the bindings between the selected synthesized analogues and the crystal structure of the human RND1 GTPase in the active GTP bound state (ID: 2CLS) with resolution 2.31 Å was retrieved from RCSB for further study [59]. The docking style involved adding hydrogen and removing both water and hetero atoms.

4. Conclusion

This study aimed to develop new anticancer agents with enhanced activity and reduced side effects. Various azolopyrimidine derivatives linked to the thiazole moiety were synthesized, including pyrazolopyrimidine, triazolopyrimidine, isoxazole, and pyrimidinethione compounds. A total of 20 novel compounds were produced and evaluated for their cytotoxic effects against HSV-1, HIV-1, and different cancer cell lines. Among these, compounds **5**, **7**, and **9** demonstrated the strongest anticancer activity. Molecular docking studies supported these findings by revealing strong binding affinities for these derivatives. Overall, the study successfully synthesized promising compounds with notable anticancer potential.

CRediT authorship contribution statement

Fatmah O. Sefrji: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Formal analysis. **Abdulmajeed F. Alrefaei:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Mohammed A. Imam:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Data curation. **Gadeer R.S. Ashour:** Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Methodology. **Matokah M. Abualnaja:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Roba M.S. Attar:** Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Methodology, Formal analysis. **A.A.A. Darwish:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Nashwa M. El-Metwaly:** Writing – review & editing, Supervision, Project administration.

Consent to participate

All authors participated directly in the current research work.

Consent to publish

The authors agree to publish the article under the Creative Commons Attribution License.

Ethical approval

Not applicable.

Availability of data and materials

The data included in article/supplementary material/references in the article.

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

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