Abstract: Pyridine, 1,3,4-thiadiazole, and 1,3-thiazole derivatives have various biological activities, such as antimicrobial, analgesic, anticonvulsant, and antitubercular, as well as other anticipated biological properties, including anticancer activity. The starting 1-(3-cyano-4,6-dimethyl-2-oxopyridin-1(2H)-yl)-3-phenylthiourea (2) was prepared and reacted with various hydrazonoyl halides 3a–h, α-haloketones 5a–d, 3-chloropentane-2,4-dione 7a, and ethyl 2-chloro-3-oxobutanoate 7b, which afforded the 3-aryl-5-substituted 1,3,4-thiadiazoles 4a–h, 3-phenyl-4-arylthiazoles 6a–d and the 4-methyl-3-phenyl-5-substituted thiazoles 8a,b, respectively. The structures of the synthesized products were confirmed by spectral data. All of the compounds also showed remarkable anticancer activity against the cell line of human colon carcinoma (HTC-116) as well as hepatocellular carcinoma (HepG-2) compared with the Harmine as a reference under in vitro condition. 1,3,4-Thiadiazole 4h was found to be most promising and an excellent performer against both cancer cell lines (IC_{50} = 2.03 ± 0.72 and 2.17 ± 0.83 µM, respectively), better than the reference drug (IC_{50} = 2.40 ± 0.12 and 2.54 ± 0.82 µM, respectively). In order to check the binding modes of the above thiadiazole derivatives, molecular docking studies were performed that established a binding site with EGFR TK.

Keywords: pyridines; 1,3,4-thiadiazoles; 1,3-thiazoles; hydrazonoyl halides; molecular docking; anticancer activity

1. Introduction

Designing new, effective, selective, highly potent, although more tolerant, anticancer drugs through the identification of novel structures remains a considerable challenge for the researchers in the field of medicinal chemistry. Hybrid drug design has emerged during the past few years as a leading technique for the creation of innovative anticancer medicines that, in theory, can address many of the pharmacokinetic drawbacks of conventional anticancer medications [1]. Medical researchers have focused on pyridines, 1,3,4-thiadiazole and 1,3-thiazole systems that have led to somewhat more effective and promising results in recent years. To name just a few, the following pyridine-based small compounds have
received approval as anticancer medications: Vismodegib III, Crizotinib IV, Regorafenib II, and Sorafenib I (Figure 1) [2–4]. Different pyridine derivatives were studied for a variety of human cancer cell lines as a tool for novel anticancer drugs through the topoisomerase inhibitory activity. These results show various reports regarding different derivatives, such as bioisosteres of α-terthiophene as a potent for protein kinase C inhibitor [5], promising topoisomerase I and/or II inhibitory activity, as well as cytotoxicity against a variety of human cancer cell lines [6–10].

Among those, 1,3,4-thiadiazoles gained substantial interest due to their widespread biological activity, including antimicrobial, anti-inflammatory, antithrombotic, antihypertensive, antituberculosis, anesthetic, anticonvulsant and antiulcer activities [11–16]. Furthermore, different researchers also particularly report 1, 3, 4-thiadiazole derivatives for their excellent anticancer activity, which are confirmed by desirable IG$_{50}$ and IC$_{50}$ values in inhibitory effect, such as Filanesib and compounds I–III (Figure 1) [16–20].

1,3-Thiazoles, which derived from thiosemicarbazone derivatives, is also known for its various pharmacological applications, as its scaffold is useful for several natural, non-natural and semi-synthetic drugs, including anti-inflammatory, anti-parasitic and antineoplastic properties [13,21–31]. Numerous studies suggested that medications such as Tiazofurin, Dasatinib, and Dabrafenib that contain thiazoles may have anticancer properties against different cancer types (Figure 1) [32–34].

![Figure 1](image_url)

Figure 1. Examples of anticancer drugs bearing pyridine, thiazole, and thiadiazole moieties.

Under the influence of the findings mentioned above, continuous efforts were performed to synthesize innovative anticancer compounds [13,35–46]. The present report aims to elaborate on the new series of thiadiazole-pyridines as well as thiazole-pyridines that might have cytotoxic effects via the inhibition of protein Epidermal Growth Factor Tyrosine Kinase receptor (EGFR TK), which plays an essential mediating role in cell proliferation, angiogenesis, apoptosis, and metastatic spread compared with reference drugs.

2. Results and Discussion

1-(3-Cyano-4,6-dimethyl-2-oxopyridin-1(2H)-yl)-3-phenylthiourea (2) [47] was synthesized through the reaction of phenyl isothiocyanate with 1-amino-4,6-dimethyl-2-oxo-
1,2-dihydropyridine-3-carbonitrile (1) under the influence of a catalytic amount of KOH in absolute ethanol as a solvent, as shown in Scheme 1.

The conduct of compound 2 towards various hydrazonoyl chlorides was explored to synthesize a novel series of 1,3,4-thiadiazole derivatives. Therefore, when compound 2 was treated with hydrazonoyl chlorides 3a–h in ethanol as a solvent in the presence of a TEA as a catalyst, it afforded a series of single product, recognized as 4,6-dimethyl-2-oxo-1-(3-aryl-5-substituted-1,3,4-thiadiazol-2(3H)-ylidene)amino)-1,2-dihydropyridine-3-carbonitriles 4a–h (Scheme 1). The 1,3,4-thiadiazole 4 was formed through the alkylation of the thiol group present in thiosemicarbazone moiety, an intramolecular cyclization and finally elimination of aniline molecule.

All the structures of products 4a–h were confirmed by elemental analyses followed by spectral data. In general, the 1H-NMR spectra of 4a (see Supplementary Materials), taken as an example, showed a singlet (1H) at δ 6.39 ppm corresponding to the pyridine proton, three singlets at δ 2.01, 2.33 and 2.46 ppm corresponding to the three CH groups and a multiplet δ 7.09–7.46 ppm corresponding to the five aromatic protons. The 13C-NMR spectrum of 4a revealed two signals at δ = 25.6, 194.7 ppm, which is characteristic for the acetyl group (CH$_3$C=O). The disappearance of the two NH absorption bands was also observed in the IR spectra because of the elimination of amine groups from the starting material 2. Moreover, the mass spectrum of the products 4a–h revealed a molecular ion peaks at the expected m/z values.

![Scheme 1. Synthesis of thiadiazoles 4a–h.](image_url)

In addition to this, the chemical reactivity of compound 2 with several of α-haloketones was also investigated to synthesize a series of novel thiazole derivatives. Accordingly, compound 2 reacted with α-haloketones 5a–d in the presence of TEA as a catalyst under refluxing condition using EtOH as a solvent that resulted a corresponding thiazoles 6a–d series, as shown in Scheme 2 (see Experimental).

All the structures of the series of products 6a–e were also confirmed through the analytical followed by spectral data analysis (see Experimental). Compound 6c showed a typical singlet signal that appeared at δ 3.82, 6.55, and 6.80 ppm due to the OCH$_3$ group, pyridine-H$_5$, and thiazole-H$_5$, respectively, in the 1H-NMR spectra. In addition to this, a multiplet is observed in the region: 7.01–7.47 ppm assignable to the nine aromatic hydrogens. On the other hand, the 13C-NMR spectrum of 6c showed four signals at δ = 17.8, 21.2, 56.7 and 162.4 ppm characteristic for 2 Ar-CH$_3$, Ar-OCH$_3$ and C=O groups, respectively, in addition to sixteen aromatic carbon signals in the range of 107.3–149.4 ppm.
Finally, compound 2 reacted with α-chloro compounds 7a,b under refluxing condition in the presence of EtOH as a solvent and TEA as a catalyst that resulted a single product, identified as 4,6-dimethyl-1-((4-methyl-3-phenyl-5-substitutedthiazol-2(3H)-ylidene) amino)4yridine-2(1H)-ones 8a,b, as outlined in Scheme 2.

The structure of the isolated product 8 was inferred from its IR and 1H-NMR spectral data and elemental analysis (see Experimental).

2.1. Anti-Cancer Activity

The series of prepared compounds 4a–h and 6a–d were investigated against human colon carcinoma (HCT-116) followed by the hepatocellular carcinoma (HepG2) cell lines to obtain pharmacological activities using Harmine as a reference drug through colorimetric MTT assay under in vitro conditions. The survival curve was obtained by plotting the relation between the concentrations of the drugs against the surviving cells, resulting in the 50% inhibitory concentration (IC50). The anti-proliferative activity is also achieved through the expression of the mean IC50 by three independent experiments (µM) ± standard deviation calculated from three replicates.

Table 1 and Figure 2 summarize the structure- and concentration-dependent anticancer activities of the series of compounds against HTC-116 cell lines. An in vitro inhibition activity shows a positive trend along with all tested compounds. Compounds such as 4c, 4d, 4f and 4g show comparable activity to that of Harmine (IC50 = 2.40 ± 0.12 µM) as a reference, whereas compound 4h demonstrates even better results compared with the same reference. A similar trend of results was also observed for the hepatocellular carcinoma (HepG2) cell line assay, where 4c, 4d, 4f, 4g and 4h show either comparable or improved inhibitory activity, with 6h (IC50 = 2.17 ± 0.83 µM) showing the maximum effect in comparison with the reference Harmine (IC50 = 2.54 ± 0.82 µM).
Table 1. The anticancer activity of the series of compounds 4a–h, and 6a–d towards human colon carcinoma (HCT-116) and hepatocellular carcinoma (HepG2) cell lines expressed as IC₅₀ values (µM) ± standard deviation from three replicates.

| Tested Compounds | R          | X (or Y) | IC₅₀ (µM)         |
|------------------|------------|----------|------------------|
|                  |            |          | HCT-116          | HepG2          |
| 4a               | COCH₃      | H        | 13.39 ± 1.04     | 16.44 ± 1.06   |
| 4b               | COCH₃      | CH₃      | 32.57 ± 2.37     | 37.56 ± 1.24   |
| 4c               | COCH₃      | Cl       | 7.91 ± 0.83      | 9.18 ± 0.91    |
| 4d               | COCH₃      | 2,4-diCl | 5.04 ± 0.59      | 7.32 ± 0.75    |
| 4e               | COOEt      | CH₃      | 16.25 ± 1.05     | 19.35 ± 1.30   |
| 4f               | COOEt      | Cl       | 4.37 ± 0.28      | 6.94 ± 0.69    |
| 4g               | COOEt      | NO₂      | 3.35 ± 0.46      | 3.94 ± 0.80    |
| 4h               | CONHPh     | H        | 2.03 ± 0.72      | 2.17 ± 0.83    |
| 6a               | –          | H        | 15.57 ± 1.30     | 19.12 ± 1.36   |
| 6b               | –          | CH₃      | 36.29 ± 1.32     | 25.90 ± 0.70   |
| 6c               | –          | OCH₃     | 21.00 ± 1.28     | 19.37 ± 1.29   |
| 6d               | –          | Cl       | 9.61 ± 0.88      | 7.36 ± 0.85    |
| Harmine          | –          | –        | 2.40 ± 0.12      | 2.54 ± 0.82    |

For thiadiazoles 4a–h: 4h (amidophenyl, has a phenyl ring along with electron withdrawing amido group resulting strongest activity) > 4g (strong electron withdrawing nitro group, increases activity) > 4f (with ester group along with one electron withdrawing Cl atom) > 4d (acetyl group with electron withdrawing 2 Cl atom) > 4c (acetyl group with mild electron withdrawing one Cl atom) > 4a (with acetyl group with un-substituted phenyl group) > 4e (ester with methyl group) > 4b (acetyl group with methyl group, electron
The anticancer activity of series of compounds 4a–h (Figures 2 and 3). Overall, electron donating groups decrease activity). Overall, electron releasing groups decrease the activity, whereas strong electron withdrawing groups increase the activity. A selective high activity is observed, particularly with 4h, possibly due to the fact that 4h possess one extra phenyl ring connected with the pyridine group in the amino side, which significantly enhances its aromatic π-π interaction with the Phe, Tyr and Trp residues. This is in coherence with the harmine where one phenyl and one pyridine is fused with an indole group, resulting in the same kind of interaction with amino acid residues containing an aromatic group. In addition to this, a noteworthy mention would be, in 4h, the nitrogen in the amido group is engaged in a tautomeric structure, thereby restricting the electron releasing power of nitrogen in the phenyl ring. Such interactions are absent for the rest of thiadiazole derivatives 4a–g, where only electron withdrawing group is present in the lone available phenyl ring.

This is in analogous with the thiazoles derivatives 6a–d, where only electron releasing groups are present with the single phenyl group available, resulting in less activity compared to thiadiazoles 4a–h with the only exception of 6d (an electron withdrawing Cl atom is present), where moderate anticancer activity is detected (Figure 3).

**Figure 3.** The anticancer activity of series of compounds 6a–d against HCT-116 and HepG2 cell lines.

### 2.2. Docking Study for Cytotoxicity

The protein Epidermal Growth Factor Receptor Tyrosine Kinase Domain (EGFR TK) was selected for this study, where the ability to inhibit this receptor ultimately leads to the blockade of the growth pathway, giving a promising anti-cancer agent [48]. The lower binding energy resulting from the association of the compound with the targeted protein is an indication of a higher binding efficiency. The results of the docking protocol were validated by the re-docking of the co-crystallized ligand (W19) inside the active site of EGFR TK (Figures 4 and 5). Harmine was used in this study as an EGFR TK inhibitor. By comparing the binding affinity of different screened synthesized compounds with Harmine (ΔG of –7.1), it was found that compound 4h showed the best binding affinity with ΔG of –10.8, and 4b, 4c, 4d, 4e, 4f, 6a, 6b, and 6c showed binding activity ΔG – 8.1 to –9.2. The screened compounds showed a possible interaction with EGFR TK active sites as depicted in Table 2 and Figures 6–13.
Figure 4. 3D molecular interactions of re-docked co-crystalized ligand (W19) with EGFR TK residues. The hydrogen bonds are represented as green dashed lines; the pi interactions are represented as orange lines.

Figure 5. Two-dimensional molecular interactions of re-docked co-crystalized ligand (W19) with EGFR TK residues.
Figure 5. Two-dimensional molecular interactions of re-docked co-crystalized ligand (W19) with EGFR TK residues.

Figure 6. 3D molecular interactions of Harmine reference drug with EGFR TK residues. The hydrogen bonds are represented as green dashed lines; the pi interactions are represented as orange lines.

Figure 7. Two-dimensional molecular interactions of Harmine reference drug with EGFR TK residues.

Figure 8. 3D molecular interactions of compound 4h with EGFR TK residues. The hydrogen bonds are represented as green dashed lines; the pi interactions are represented as orange lines.

Figure 9. 3D molecular interactions of compound 4i with EGFR TK residues. The hydrogen bonds are represented as green dashed lines; the pi interactions are represented as orange lines.

Figure 10. 3D molecular interactions of compound 4j with EGFR TK residues. The hydrogen bonds are represented as green dashed lines; the pi interactions are represented as orange lines.

Figure 11. 3D molecular interactions of compound 4k with EGFR TK residues. The hydrogen bonds are represented as green dashed lines; the pi interactions are represented as orange lines.

Figure 12. 3D molecular interactions of compound 4l with EGFR TK residues. The hydrogen bonds are represented as green dashed lines; the pi interactions are represented as orange lines.
Figure 7. Two-dimensional molecular interactions of Harmine reference drug with EGFR TK residues.

Figure 8. 3D molecular interactions of compound $4h$ with EGFR TK residues. The hydrogen bonds are represented as green dashed lines; the pi interactions are represented as orange lines.

Figure 9. Two-dimensional molecular interactions of compound $4h$ with EGFR TK residues.

Figure 10. Mapping surface showing compound $4h$ occupying the active pocket of EGFR TK.
Figure 9. Two-dimensional molecular interactions of compound 4h with EGFR TK residues.

Figure 10. Mapping surface showing compound 4h occupying the active pocket of EGFR TK.

Figure 11. 3D molecular interactions of compound 6b with EGFR TK residues. The hydrogen bonds are represented as green dashed lines; the pi interactions are represented as orange lines.

Figure 12. Two-dimensional molecular interactions of compound 6b with EGFR TK residues.
Figure 11. 3D molecular interactions of compound 6b with EGFR TK residues. The hydrogen bonds are represented as green dashed lines; the pi interactions are represented as orange lines.

Figure 12. Two-dimensional molecular interactions of compound 6b with EGFR TK residues.

Figure 13. Mapping surface showing compound 6b occupying the active pocket of EGFR TK.
Table 2. The binding scores and interactions of the examined compounds and the Harmine inhibitor inside the binding pocket of receptor of (3W33) for EGFR TK.

| Compounds | Binding Scores (kcal/mol) | Hydrogen Bond Interactions | Distance (Å) | Hydrophobic Interactions | Distance (Å) |
|-----------|--------------------------|-----------------------------|--------------|--------------------------|--------------|
| 4a        | −8.5                     | MET769                      | 2.08         | LEU694                   | 3.78         |
|           |                          |                             |              | VAL702                   | 3.75         |
|           |                          |                             |              | LYS721                   | 3.46, 3.93   |
|           |                          |                             |              | LEU820                   | 3.24, 3.90   |
|           |                          |                             |              | THR830                   | 3.77         |
| 4b        | −9.2                     | MET769                      | 1.96         | LEU694                   | 3.74         |
|           |                          |                             |              | VAL702                   | 3.69         |
|           |                          |                             |              | ALA719                   | 3.89         |
|           |                          |                             |              | LYS721                   | 3.48, 3.71   |
|           |                          |                             |              | LEU764                   | 3.77         |
|           |                          |                             |              | LEU820                   | 3.39         |
| 4c        | −8.9                     | MET769                      | 2.07         | LEU694                   | 3.78         |
|           |                          |                             |              | VAL702                   | 3.69         |
|           |                          |                             |              | ALA719                   | 3.87         |
|           |                          |                             |              | LYS721                   | 3.47         |
|           |                          |                             |              | LEU820                   | 3.21, 3.94   |
|           |                          |                             |              | THR830                   | 3.75         |
| 4d        | −9.2                     | GLU738, THR830, ASP831, PHE832 | 2.71, 2.64, 2.20, 3.17 | LEU694                   | 3.55         |
|           |                          |                             |              | VAL702                   | 3.66         |
|           |                          |                             |              | ALA719                   | 3.04         |
|           |                          |                             |              | LYS721                   | 3.56         |
|           |                          |                             |              | LEU764                   | 3.95         |
|           |                          |                             |              | THR766                   | 3.59         |
|           |                          |                             |              | LEU820                   | 3.50         |
|           |                          |                             |              | PHE699                   | 3.58         |
| 4e        | −8.7                     | GLU738, THR830, ASP831, PHE832 | 2.71, 2.64, 2.20, 3.17 | LEU694                   | 3.55         |
|           |                          |                             |              | VAL702                   | 3.66         |
|           |                          |                             |              | ALA719                   | 3.04         |
|           |                          |                             |              | LYS721                   | 3.56         |
|           |                          |                             |              | LEU764                   | 3.95         |
|           |                          |                             |              | THR766                   | 3.59         |
|           |                          |                             |              | LEU820                   | 3.58         |
|           |                          |                             |              | PHE699                   | 3.58         |
| 4f        | −8.8                     | MET769                      | 2.10         | LEU694                   | 3.88         |
|           |                          |                             |              | VAL702                   | 3.58         |
|           |                          |                             |              | ALA719                   | 3.87         |
|           |                          |                             |              | LYS721                   | 3.42         |
|           |                          |                             |              | LEU820                   | 3.33, 3.86   |
|           |                          |                             |              | THR830                   | 3.92         |
| 4g        | −9.1                     | MET769                      | 2.56         | LEU694                   | 3.74         |
|           |                          |                             |              | VAL702                   | 3.38         |
|           |                          |                             |              | ALA719                   | 3.68         |
|           |                          |                             |              | LYS721                   | 3.59         |
|           |                          |                             |              | LEU820                   | 3.31, 3.81   |
| 4h        | −10.8                    | ARG841, ASN842, LYS745, THR854 | 2.08, 2.27, 3.48, 3.45 | LEU718                   | 3.74, 3.50   |
|           |                          |                             |              | VAL726                   | 3.61, 3.30   |
|           |                          |                             |              | ALA743                   | 3.67         |
|           |                          |                             |              | LYS745                   | 3.36, 3.89   |
|           |                          |                             |              | LEU788                   | 3.66         |
|           |                          |                             |              | THR790                   | 3.80         |
| Compounds | Binding Scores (kcal/mol) | Hydrogen Bond Interactions | Distance (Å) | Hydrophobic Interactions | Distance (Å) |
|-----------|--------------------------|---------------------------|-------------|--------------------------|-------------|
| 6a        | −8.8                     |                           |             | LEU694                   | 3.69        |
|           |                          |                           |             | VAL702                   | 3.13, 3.91  |
|           |                          |                           |             | ALA719                   | 3.61        |
|           |                          |                           |             | LYS721                   | 3.73, 3.87  |
|           |                          |                           |             | LEU820                   | 3.41, 3.64  |
|           |                          |                           |             | LEU718                   | 3.90, 3.39, 3.47 |
|           |                          |                           |             | VAL726                   | 3.64        |
|           |                          |                           |             | ASP855                   | 3.67        |
|           |                          |                           |             | LEU777                   | 3.08        |
|           |                          | CYS797                    | 2.29        | LEU788                   | 3.48        |
|           |                          |                           |             | THR790                   | 3.48        |
|           |                          |                           |             | LEU844                   | 3.53        |
|           |                          |                           |             | PHE997                   | 3.29        |
| 6b        | −8.9                     |                           |             | LEU694                   | 3.39        |
|           |                          |                           |             | VAL702                   | 3.32, 3.51  |
|           |                          |                           |             | LYS721                   | 3.69, 3.73  |
|           |                          |                           |             | LEU820                   | 3.39        |
| 6c        | −7.9                     | THR766                    | 2.38        | PHE699                   | 3.57        |
|           |                          |                           |             | VAL702                   | 3.95        |
|           |                          |                           |             | ALA719                   | 3.50        |
|           |                          |                           |             | MET769                   | 3.93        |
|           |                          |                           |             | ARG817                   | 3.78        |
|           |                          |                           |             | LEU820                   | 3.91, 3.48  |
| 6d        | −8.8                     | THR766                    | 2.58        | LEU718                   | 3.61        |
|           |                          |                           |             | VAL726                   | 3.58, 3.64  |
|           |                          |                           |             | LYS745                   | 3.76        |
|           |                          |                           |             | THR790                   | 3.63        |
|           |                          |                           |             | LEU792                   | 3.72        |
| Harmine   | −7.1                     |                           |             | LEU718                   | 3.67        |
|           |                          |                           |             | VAL726                   | 3.65, 3.94  |
|           |                          |                           |             | LYS745                   | 3.85        |
|           |                          |                           |             | LEU777                   | 3.79        |
|           |                          |                           |             | LEU788                   | 3.97        |
|           |                          |                           |             | THR790                   | 3.73        |
|           |                          |                           |             | THR845                   | 3.79        |
| W19       | −10.8                    | LYS745                    | 2.36        | LEU718                   | 3.67        |
|           |                          |                           |             | VAL726                   | 3.65, 3.94  |
|           |                          |                           |             | LYS745                   | 3.85        |
|           |                          |                           |             | LEU777                   | 3.79        |
|           |                          |                           |             | LEU788                   | 3.97        |
|           |                          |                           |             | THR790                   | 3.73        |
|           |                          |                           |             | THR845                   | 3.79        |

### 3. Experimental

Elementar vario LIII CHNS analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany) is used to measure all elemental analysis. Electrothermal IA 9000 series Digital Melting Point Apparatus (Shanghai Jiahang Instruments Co., Jiading District, Shanghai, China) was used to obtain melting points data. Shimadzu FTIR 8101 PC infrared spectrophotometers (Shimadzu Co., Kyoto, Japan) were used to record IR spectra data in KBr discs on Pye Unicam SP 3300. Varian Mercury VX-300 NMR spectrometer (Bruker Biospin, Karlsruhe, Germany) was used with the operating frequency of 300 MHz ($^1$H-NMR) in deuterated dimethylsulfoxide (DMSO-<em>d6</em>) solvent to record NMR spectra, where chemical shifts were related to the solvent used. Shimadzu GCeMS-QP1000 EX mass spectrometer (Shimadzu Co., Kyoto, Japan) was used to record mass spectra at 70 eV. The cytotoxicity of the prepared compounds was measured by the Regional Center for Mycology and Biotechnology in Al-Azhar University, Cairo, Egypt.
Synthesis of 1-(3-cyano-4,6-dimethyl-2-oxopyridin-1(2H)-yl)-3-phenylthiourea (2)

A mixture of 1-amino-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (I) (1.63 g, 10 mmol), KOH (0.56 g, 10 mmol) in DMF (30 mL), was stirred for 10 min. Then, PhNCS (1.35 g, 10 mmol) is added under stirring condition and continued for the next 6 h. Afterwards, the solution was diluted with 30 mL of distilled water, followed by neutralization by adding aqueous AcOH dropwise, resulting in a solid recrystallized from dioxin to obtain yellowish brown crystals (71%) as a pure product of compound 2; mp = 209–211 °C (Lit mp = 205–207 °C [47]), $^{1}$H-NMR (DMSO-$d_6$): $\delta$ 2.22 (s, 3H, CH$_3$), 2.30 (s, 3H, CH$_3$), 6.34 (s, 1H, Pyridine-H5), 7.32–7.69 (m, 5H, Ar-H), 8.55 (s, br, 1H, NH), 8.95 (s, br, 1H, NH) ppm; IR (KBr): $v$ 3372, 3241 (2NH), 3033, 2951 (CH), 2218 (CN), 1675 (C=O), 1599 (C=N), 1335 (C=S) cm$^{-1}$; MS m/z (%): 298 (M$^+$, 85). Anal. Calcd for C$_{15}$H$_{14}$N$_4$OS (298.36): C, 60.38; H, 4.73; N, 18.78. Found: C, 60.24; H, 4.59; N, 18.58%.

Synthesis of 4,6-dimethyl-2-oxo-1-((3-aryl-5-substituted-1,3,4-thiadiazol-2(3H)-ylidene) amino)-1,2-dihydropyridine-3-carbonitriles (4a–h).

A mixture of compound 2 (0.298 g, 1 mmol) and appropriate hydrazonyl halides 3a–h (1 mmol) in DMF (20 mL) containing Et$_3$N (0.1 g, 1 mmol) was heated under reflux for 3–6 h. The resultant solid product was recrystallized by appropriate solvent to give thiadiazoles 4a–h. Below is a list of the spectrum information and physical characteristics of the products 4a–h.

1-(((5-Acetyl-3-(4-chlorophenyl)-1,3,4-thiadiazol-2(3H)-ylidene)amino)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (4a).

Yellow solid (79%); m.p. 233–235 °C (DMF); $^{1}$H-NMR (DMSO-$d_6$): $\delta$ 2.01 (s, 3H, CH$_3$), 2.33 (s, 3H, CH$_3$), 2.46 (s, 3H, CH$_3$), 6.39 (s, 1H, Pyridine-H5), 7.09–7.46 (m, 5H, Ar-H) ppm; $^{13}$C-NMR (DMSO-$d_6$): $\delta$ 19.6, 21.2, 25.6 (3CH$_3$), 107.8, 115.9, 116.4, 116.8, 122.7, 123.1, 124.0, 125.8, 138.1, 142.3, 152.3 (Ar-C and C=N), 163.2, 194.7 (2 C=O) ppm; IR (KBr): $v$ 3047, 2933 (CH), 2220 (CN), 1704, 1651 (C=O), 1599 (C=N) cm$^{-1}$; MS m/z (%): 365 (M$^+$, 49). Anal. Calcd. for C$_{18}$H$_{15}$N$_5$S (365.41): C, 59.17; H, 4.14; N, 19.17. Found C, 59.30; H, 4.04; N, 19.11%.

1-(((5-Acetyl-3-phenyl-1,3,4-thiadiazol-2(3H)-ylidene)amino)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (4b).

Yellow solid (80%); m.p. 243–245 °C (DMF); $^{1}$H-NMR (DMSO-$d_6$): $\delta$ 2.01 (s, 3H, CH$_3$), 2.32 (s, 3H, CH$_3$), 2.40 (s, 3H, CH$_3$), 2.45 (s, 3H, CH$_3$), 6.39 (s, 1H, Pyridine-H5), 7.02–7.80 (m, 4H, Ar-H) ppm; $^{13}$C-NMR (DMSO-$d_6$): $\delta$ 19.1, 20.7, 21.2, 25.4 (4CH$_3$), 107.3, 116.5, 122.9, 127.5, 130.1, 130.6, 133.8, 138.2, 145.2, 154.2 (Ar-C and C=N), 162.1, 194.2 (2 C=O) ppm; IR (KBr): $v$ 3028, 2940 (CH), 2217 (CN), 1703, 1667 (C=O), 1597 (C=N) cm$^{-1}$; MS m/z (%): 379 (M$^+$, 38). Anal. Calcd. for C$_{19}$H$_{17}$N$_5$O$_2$S (379.44): C, 60.14; H, 4.52; N, 18.46. Found C, 60.05; H, 4.42; N, 18.29%.

1-(((5-Acetyl-3-(p-tolyl)-1,3,4-thiadiazol-2(3H)-ylidene)amino)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (4c).

Yellow solid (78%); m.p. 228–230 °C (dioxane); $^{1}$H-NMR (DMSO-$d_6$): $\delta$ 2.03 (s, 3H, CH$_3$), 2.25 (s, 3H, CH$_3$), 2.41 (s, 3H, CH$_3$), 6.39 (s, 1H, Pyridine-H5), 7.11–7.85 (m, 4H, Ar-H) ppm; IR (KBr): $v$ 3052, 2944 (CH), 2218 (CN), 1709, 1663 (C=O), 1598 (C=N) cm$^{-1}$; MS m/z (%): 401 (M$^+$, 2, 31), 399 (M$^+$, 100). Anal. Calcd. for C$_{19}$H$_{14}$ClN$_5$O$_2$S (399.85): C, 54.07; H, 3.53; N, 17.52. Found C, 54.01; H, 3.36; N, 17.48%.

1-(((5-Acetyl-3-(2-chlorophenyl)-1,3,4-thiadiazol-2(3H)-ylidene)amino)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (4d).

Brown solid (79%); m.p. 262–264 °C (DMF); $^{1}$H-NMR (DMSO-$d_6$): $\delta$ 2.04 (s, 3H, CH$_3$), 2.31 (s, 3H, CH$_3$), 2.43 (s, 3H, CH$_3$), 6.39 (s, 1H, Pyridine-H5), 7.26–7.86 (m, 3H, Ar-H) ppm; IR (KBr): $v$ 3047, 2937 (CH), 2222 (CN), 1711, 1672 (C=O), 1601 (C=N) cm$^{-1}$; MS m/z (%): 434 (M$^+$, 81). Anal. Calcd. for C$_{18}$H$_{13}$Cl$_2$N$_5$O$_2$S (434.30): C, 49.78; H, 3.02; N, 16.13. Found C, 49.83; H, 3.00; N, 16.04%.
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Ethyl 5-((3-cyano-4,6-dimethyl-2-oxopyridin-1(2H)-yl)imino)-4-(p-tolyl)-4,5-dihydro-1,3,4-thiadiazole-2-carboxylate (4e).

Yellow solid (77%); m.p. 189–191 °C (EtOH\DMF); $^1$H-NMR (DMSO-$d_6$): $\delta$ 1.17–1.21 (t, 3H, CH$_3$), 2.01 (s, 3H, CH$_3$), 2.32 (s, 3H, CH$_3$), 2.42 (s, 3H, CH$_3$), 4.13–4.17 (q, 2H, CH$_2$), 6.45 (s, 1H, Pyridine-H5), 7.12–7.59 (m, 4H, Ar-H) ppm; $^{13}$C-NMR (DMSO-$d_6$): $\delta$ 12.9, 17.5, 21.1, 21.7 (4CH$_3$), 117.3, 119.0, 121.2, 121.7, 122.4, 124.1, 125.0, 130.1, 139.4, 142.5, 151.2 (Ar-C and C=N), 161.2, 163.5 (2 C=O) ppm; IR (KBr): $\nu$ 3049, 2935 (CH), 2219 (CN), 1723, 1669 (C=O), 1600 (C=N) cm$^{-1}$; MS m/z (%): 409 (M$^+$, 14). Anal. Calcd. for C$_{20}$H$_{19}$N$_5$O$_3$S (409.46): C, 58.67; H, 4.68; N, 17.10. Found C, 58.52; H, 4.55; N, 17.02%.

Ethyl 4-(4-chlorophenyl)-5-((3-cyano-4,6-dimethyl-2-oxopyridin-1(2H)-yl)imino)-4,5-dihydro-1,3,4-thiadiazole-2-carboxylate (4f).

Yellow solid (77%); m.p. 185–187 °C (EtOH); $^1$H-NMR (DMSO-$d_6$): $\delta$ 1.20–1.27 (t, 3H, CH$_3$), 2.03 (s, 3H, CH$_3$), 2.38 (s, 3H, CH$_3$), 4.11–4.17 (q, 2H, CH$_2$), 6.46 (s, 1H, Pyridine-H5), 7.23–7.60 (m, 4H, Ar-H) ppm; IR (KBr): $\nu$ 3046, 2937 (CH), 2217 (CN),1722, 1660 (C=O), 1601 (C=N) cm$^{-1}$; MS m/z (%): 431 (M$^+$, 2, 20), 429 (M$^+$, 63). Anal. Calcd. for C$_{19}$H$_{18}$ClIN$_5$O$_3$S (429.88): C, 53.09; H, 3.75; N, 16.29. Found C, 53.18; H, 3.58; N, 16.14%.

Ethyl 5-((3-cyano-4,6-dimethyl-2-oxopyridin-1(2H)-yl)imino)-4-(4-nitrophenyl)-4,5-dihydro-1,3,4-thiadiazole-2-carboxylate (4g).

Yellow solid (73%); m.p. = 217–219 °C (EtOH\DMF); $^1$H-NMR (DMSO-$d_6$): $\delta$ 1.20–1.27 (t, 3H, CH$_3$), 2.29 (s, 3H, CH$_3$), 2.42 (s, 3H, CH$_3$), 4.18–4.26 (q, 2H, CH$_2$), 6.84 (s, 1H, Pyridine-H5), 7.27–8.34 (m, 4H, Ar-H) ppm; IR (KBr): $\nu$ 3039, 2943 (CH), 2218 (CN),1719, 1665 (C=O), 1600 (C=N) cm$^{-1}$; MS m/z (%): 440 (M$^+$, 70). Anal. Calcd. for C$_{19}$H$_{18}$NI$_5$O$_3$S (440.43): C, 51.81; H, 3.66; N, 19.08. Found C, 51.66; H, 3.50; N, 19.03%.

5-((3-Cyano-4,6-dimethyl-2-oxypyridin-1(2H)-yl)imino)-N,4-diphenyl-4,5-dihydro-1,3,4-thiadiazole-2-carboxamide (4h).

Yellow solid (86%); m.p = 277–279 °C (DMF); $^1$H-NMR (DMSO-$d_6$): $\delta$ 2.16 (s, 3H, CH$_3$), 2.38 (s, 3H, CH$_3$), 6.42 (s, 1H, Pyridine-H5), 7.03–7.79 (m, 10H, Ar-H) ppm; $^{13}$C-NMR (DMSO-$d_6$): $\delta$ 17.9, 21.4 (2CH$_3$), 112.4, 115.9, 119.3, 120.5, 121.5, 121.1, 124.3, 124.7, 125.2, 128.6, 132.4, 134.6, 139.4, 141.5, 152.4 (Ar-C and C=N), 161.2, 162.7 (2 C=O) ppm; IR (KBr): $\nu$ 3278 (NH), 3061, 2947 (CH), 2219 (CN),1678, 1663 (C=O), 1597 (C=N) cm$^{-1}$; MS m/z (%): 442 (M$^+$, 18). Anal. Calcd. for C$_{23}$H$_{18}$N$_6$O$_2$S (442.50): C, 62.43; H, 4.10; N, 18.99. Found C, 62.52; H, 4.04; N, 18.75%.

Synthesis of thiazoles 6a–d or 8a,b.

A mixture of 2 (0.298 g, 1 mmol) and α-haloketones 5a–d or 3-chloropentane-2,4-dione (7a) or ethyl 2-chloro-3-oxobutanatoate (7b) (1 mmol) in DMF (15 mL) was refluxed for 4–6 h and was continuously monitored in TLC. The separation of the product was clearly observed during the course of the reaction. The resultant solid product was then filtered, washed several times with water, dried and recrystallized in the proper solvent to give the corresponding thiazoles 6a–d or 8a,b, respectively.

1-((3,4-Diphenylthiazol-2(3H)-ylidene)amino)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (6a).

Yellow solid (74%); m.p = 230–232 °C (DMF); $^1$H-NMR (DMSO-$d_6$): $\delta$ 2.23 (s, 3H, CH$_3$), 2.34 (s, 3H, CH$_3$), 6.37 (s, 1H, Pyridine-H5), 6.67 (s, 1H, Thiazole-H5), 7.19–8.05 (m, 10H, Ar-H) ppm; IR (KBr): $\nu$ 3047, 2937 (CH), 2217 (CN),1667 (C=O), 1599 (C=N) cm$^{-1}$; MS m/z (%): 398 (M$^+$, 37). Anal. Calcd. for C$_{23}$H$_{18}$N$_6$O$_2$S (398.48): C, 69.33; H, 4.55; N, 14.06. Found C, 69.17; H, 4.42; N, 14.04%.

4,6-Dimethyl-2-oxo-1-((3-phenyl-4-(p-tolyl)thiazol-2(3H)-ylidene)amino)-1,2-dihydropyridine-3-carbonitrile (6b).

Yellow solid (77%); m.p = 213–215 °C (EtOH); $^1$H-NMR (DMSO-$d_6$): $\delta$ 2.23 (s, 3H, CH$_3$), 2.24 (s, 3H, CH$_3$), 2.34 (s, 3H, CH$_3$), 6.37 (s, 1H, Pyridine-H5), 7.07 (s, 1H, Thiazole-H5),
7.33–7.44 (m, 9H, Ar-H) ppm; IR (KBr): ν 3042, 2950 (CH), 2219 (CN), 1671 (C=O), 1602 (C=N) cm⁻¹; MS m/z (%): 412 (M⁺, 18). Anal. Calcd. for C₂₃H₂₀N₄O₅ (412.51): C, 69.88; H, 4.89; N, 13.58. Found C, 69.70; H, 4.83; N, 13.37%.

1-((4-((4-Methoxyphenyl)-3-phenylthiazol-2(3H)-ylidene)amino)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (6c).

Yellow solid (80%); mp = 221–223 °C (EtOH|DMF); ¹H-NMR (DMSO-d₆): δ 2.23 (s, 3H, CH₃), 2.32 (s, 3H, CH₃), 3.82 (s, 3H, OCH₃), 6.55 (s, 1H, Pyridine-H5), 6.80 (s, 1H, Thiazole-H5), 7.01–7.47 (m, 9H, Ar-H) ppm; ¹³C-NMR (DMSO-d₆): δ 17.8, 21.2 (CH₃), 56.7 (OCH₃), 107.8, 113.9, 115.0, 118.3, 121.9, 122.5, 123.6, 130.0, 131.8, 135.6, 138.9, 140.8, 144.0, 152.0, 152.5, 154.3 (Ar-C and C=N), 162.4 (C=O) ppm; IR (KBr): ν 3074, 2928 (CH), 2218 (CN), 1683 (C=O), 1603 (C=N) cm⁻¹; MS m/z (%): 403 (M⁺, 23). Anal. Calcd. for C₂₄H₂₀N₄O₂S (428.51): C, 67.27; H, 4.70; N, 13.08. Found C, 67.36; H, 4.61; N, 13.02%.

1-((4-((4-Chlorophenyl)-3-phenylthiazol-2(3H)-ylidene)amino)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (6d).

Yellow solid (79%); mp = 217–219 °C (DMF); ¹H-NMR (DMSO-d₆): δ 2.23 (s, 3H, CH₃), 2.34 (s, 3H, CH₃), 6.76 (s, 1H, Pyridine-H5), 7.01 (s, 1H, Thiazole-H5), 7.12–7.77 (m, 9H, Ar-H) ppm; IR (KBr): ν 3048, 2927 (CH), 2218 (CN), 1669 (C=O), 1602 (C=N) cm⁻¹; MS m/z (%): 409 (M⁺ + 2, 13), 407 (M⁺, 41). Anal. Calcd. for C₂₅H₂₁ClN₄O₂S (432.93): C, 63.81; H, 3.96; N, 12.94. Found C, 63.62; H, 3.77; N, 12.73%.

1-((5-Acetyl-4-methyl-3-phenylthiazol-2(3H)-ylidene)amino)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (8a).

Yellow solid (83%); mp = 195–197 °C (EtOH); ¹H-NMR (DMSO-d₆): δ 2.18 (s, 3H, CH₃), 2.25 (s, 3H, CH₃), 2.33 (s, 3H, CH₃), 2.43 (s, 3H, CH₃), 6.36 (s, 1H, Pyridine-H5), 7.55–7.66 (m, 5H, Ar-H) ppm; IR (KBr): ν 3048, 2935 (CH), 2219 (CN), 1709, 1671 (C=O), 1601 (C=N) cm⁻¹; MS m/z (%): 353 (M⁺ + 5, 35). Anal. Calcd. for C₂₀H₁₈N₂O₅S (378.45): C, 63.47; H, 4.79; N, 14.80. Found C, 63.35; H, 4.62; N, 14.69%.

Ethyl 2-((3-cyano-4,6-dimethyl-2-oxopyridin-1(2H)-ylidino)-4-methyl-3-phenyl-2,3-dihydrothiazole-5-carboxylate (8b).

Yellow solid (85%); mp = 190–192 °C (EtOH); ¹H-NMR (DMSO-d₆): δ 1.21–1.25 (t, 3H, CH₃), 2.19 (s, 3H, CH₃), 2.19 (s, 3H, CH₃), 2.22 (s, 3H, CH₃), 2.38 (s, 3H, CH₃), 4.21–4.25 (q, 2H, CH₂), 6.39 (s, 1H, Pyridine-H5), 7.54–7.62 (m, 5H, Ar-H) ppm; ¹³C-NMR (DMSO-d₆): δ 14.5, 17.1, 21.3 (3CH₃), 62.5 (CH₂), 106.4, 113.3, 115.3, 119.8, 123.7, 124.5, 127.6, 131.5, 133.0, 140.2, 144.1, 148.7 (Ar-C and C=N), 162.5, 164.1 (2C=O) ppm; IR (KBr): ν 3037, 2943 (CH), 2218 (CN), 1724, 1668 (C=O), 1600 (C=N) cm⁻¹; MS m/z (%): 408 (M⁺, 74). Anal. Calcd. for C₂₁H₂₀N₂O₅S (408.48): C, 61.75; H, 4.94; N, 13.72. Found C, 61.63; H, 4.81; N, 13.53%.

3.1. Cytotoxic Activity

The cytotoxicity of the newly synthesized series of compounds was studied against HCT-116 and HepG2 cells using the MTT assay through an incubation period of 24 h [49,50].

Mammalian cell line: HCT-116 and HepG2 cells were collected from VACSERA Tissue Culture Unit, Cairo, Egypt.

3.2. Docking Method

The MOE 2019.012 suite (Chemical Computing Group ULC, Montreal, Canada) [51] was applied in order to carry all docking studies for the newly synthesized compounds to suggest their plausible mechanism of action as the protein Epidermal Growth Factor Receptor Tyrosine Kinase Domain (EGFR TK) inhibitors by evaluating their binding grooves and modes to compare with Harmine as a reference standard.

The newly prepared derivatives were placed into the MOE window, where they were treated to partial charge addition and energy minimization [52,53]. The produced compounds also were placed into a single database with the Harmine and saved as an MDB
file, which was then uploaded to the ligand icon during the docking process. The Protein Data Bank was used to generate an X-ray of the targeted Epidermal Growth Factor Receptor Tyrosine Kinase Domain (EGFR TK) 3W33. Available online: https://www.rcsb.org/structure/3W33 (accessed on 17 July 2022). Furthermore, it was readied for the docking process by following the previously detailed stages [54,55]. Additionally, the downloaded protein was error-corrected, 3D hydrogen-loaded and energy-minimized [56,57].

In a general docking procedure, the newly prepared derivatives were substituted for the ligand site. After modifying the default program requirements previously stated, the co-crystallized ligand site was selected as the docking site, and the docking process was started [58]. In a nutshell, the docking site was chosen using the dummy atoms method [59]. The placement and scoring procedures, respectively, Triangle matcher and London dG, were chosen. The stiff receptor was used as the scoring method, and the GBVI/WSA dG was used as the refining method, respectively, to select the top 10 poses out of a total of 100 poses for each docked molecule [60,61]. For further research, the optimal pose for each ligand with the highest score, binding mode and RMSD value was chosen. It is important to note that the applied MOE program underwent the first step of program verification by docking Harmine to its ligand binding of the prepared Target [62,63]. By obtaining a low RMSD value (1.43) between the newly created compounds with docked Harmine, a valid performance was demonstrated.

4. Conclusions

In this paper, two new series of aryl substituted novel pyridine-1,3,4-thiadiazoles, and pyridine-thiazoles were synthesized starting with 1-(3-cyano-4,6-dimethyl-2-oxopyridin-1(2H)-yl)-3-phenylthiourea and several available reagents. All the structures were confirmed through elemental and spectral analysis, where the plausible mechanistic approach for their formation was discussed. All the prepared derivatives showed effectiveness towards the inhibition of human colon carcinoma (HCT-116) as well as hepatocellular carcinoma (HepG2) cell lines through in vitro evaluation and an in silico docking study. From the obtained results, compound 4h (amidophenyl has a phenyl ring along with the electron withdrawing amido group resulting in the strongest activity) was found to be the strongest and most effective with 2.03 ± 0.72 µM against HCT-116, contributing its activity through a variety of interactions, such as hydrophobic interaction, hydrogen bonding in addition to aromatic stacking interactions with selected target (EGFR TK) pockets, compared with Harmine as a reference drug. A detailed analysis of all the series confirmed the electron withdrawing group present in the aryl substitution, resulting in the enhancement of anticancer activity, which could be promising for the future generation of new efficient anticancer drugs based on 1,3,5-thiadiazole and 1,3-thiazole derivatives.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27196368/s1, Samples of 1H-NMR and 13C-NMR spectra.

Author Contributions: Supervision, Investigation, Methodology, Resources, Formal analysis, Data curation, Funding acquisition, Writing—original draft, Writing—review & editing: S.M.G., A.S.A., J.Y.A.-H., A.S.B., H.Q., N.K.B., A.A. and S.M.I. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from corresponding author.

Acknowledgments: This research was funded by the Scientific Research Deanship at University of Ha’il—Saudi Arabia via a project number (RG-21081).

Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Samples of all compounds are available from the authors.
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