

Antiproliferative Activity of Some Newly Synthesized Substituted Nicotinamides Candidates Using Pyridine-2(1H) thione Derivatives as Synthons

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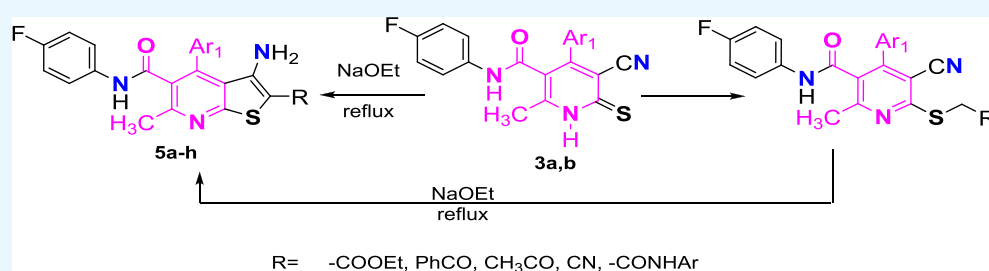


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ABSTRACT: Some new pyridinethione and thienopyridine derivatives have been synthesized and evaluated for their antiproliferative activity using the MTT assay. Nicotinamide derivatives **3** have been synthesized and used for the preparation of new condensed thieno [2,3-*b*]pyridines by their reactions with active halo compounds. Finally the synthesized thienopyridine underwent ring closure whenever possible through boiling in a solution of sodium ethoxide. The antiproliferative evaluation against (HCT-116, HepG-2, and MCF-7) human cancer cells and one human healthy cell line (BJ-1) revealed that compounds **3b**, **4c–5d**, **7b–12a**, **10d**, and **13b** have interesting antitumor activity specifically as antihepatocellular and anticolon cellular carcinoma agents. Besides, the docking results for most active derivatives were in agreement with the *in vitro* antitumor results.

1. INTRODUCTION

Development of newer, safer, and more efficient anticancer agents has a significant interest, especially the use of available chemotherapeutics is frequently limited due to unwanted side effects. Moreover, drug resistance to cancer chemotherapeutic is becoming increasingly prevalent.¹

The pyridine nucleus is predominant in numerous natural products as vitamins, coenzymes, and alkaloids (Figure 1).

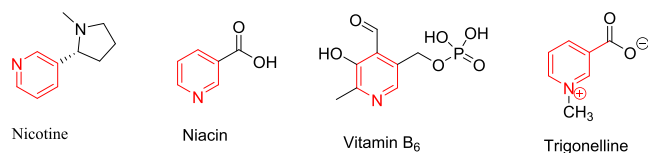


Figure 1. Natural products containing the pyridine nucleus.

Additionally, the pyridine moiety is incorporated in many drugs, including anticancer, antiviral, antimicrobial, anti-inflammatory, antihypertensive, analgesic, antimalarial and antidiabetic antioxidants, psychopharmacological antagonists, and insecticidal and antiamoebic agents.^{2–14}

Nicotinonitriles¹⁵ and pyridinethione¹⁶ were used lately by many research groups as a scaffold for synthesis of compounds

with several biological activities especially as anticancer agents. For example, 4,6-diaryl-2-oxo-1,2-dihydropyridine-3- carbonitriles have been prescribed as inhibitors of the oncogenic serine/threonine kinase PIM-1 that plays a role in the survival of cancer cells.¹⁷ In addition, an anticancer effect against MCF7, SK-OV-3 (ovarian adenocarcinoma cells), and CCRF-CEM (blood cancer cells) was detected from pyridine-2(1H)-thione derivatives.¹⁸ Also, 3-cyano-2-thioxopyridines are of great interest since they have potential synthetic and biological abilities.¹⁹

According to druglike molecules build up approach, an improvement in the pharmacological profiles may be obtained through fused analogues or by combination with other heterocycles.²⁰ Among these heterocyclic systems, thienopyridines are considered as the most active and important ones, where a number of drug containing thienopyridine nucleus are

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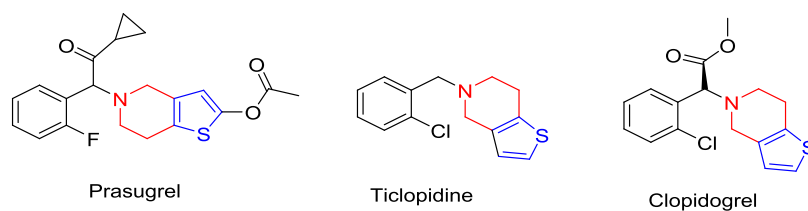
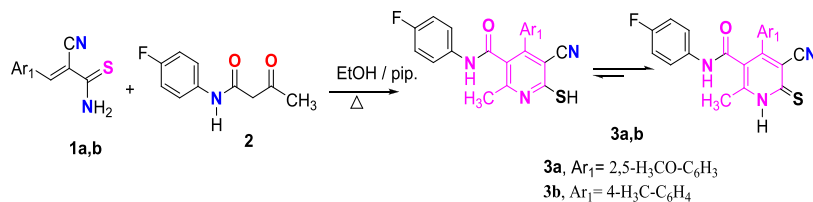
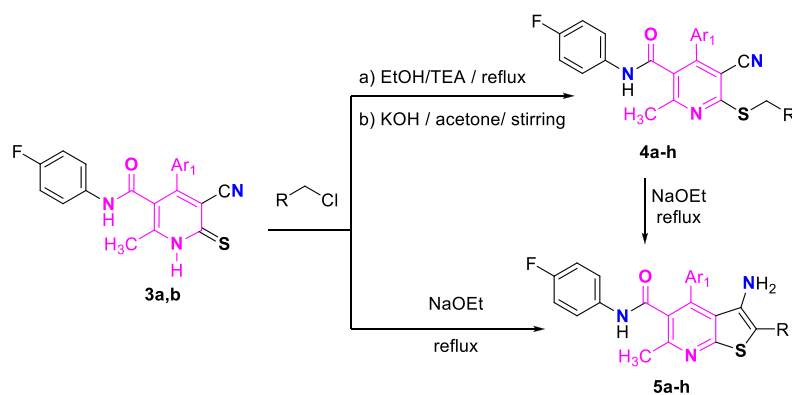


Figure 2. Marketed drugs containing a thienopyridine nucleus.

Scheme 1. Synthesis of New Cyanopyridinethione Derivatives

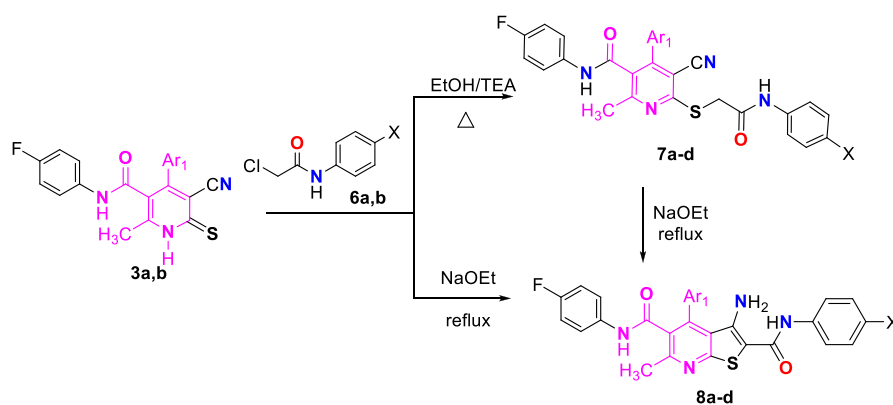


Scheme 2. Synthesis of S-Alkylated Cyanopyridines and Their Thienopyridines



Compounds 4,5, a: R = -COOEt, Ar₁ = 2,5-(CH₃O)₂-C₆H₃; b: R = -COOEt, Ar₁ = 4-H₃C-C₆H₄; c: R = PhCO, Ar₁ = 2,5-(CH₃O)₂-C₆H₃; d: R = PhCO-, Ar₁ = 4-H₃C-C₆H₄; e: R = CH₃CO, Ar₁ = 2,5-(CH₃O)₂-C₆H₃; f: R = CH₃CO, Ar₁ = 4-H₃C-C₆H₄; g: R = CN, Ar₁ = 2,5-(CH₃O)₂-C₆H₃; h: R = CN, Ar₁ = 4-H₃C-C₆H₄

Scheme 3. Synthesis of 2-(N-Aryl)carboxamidomethylthienopyridine Derivatives 7a–7d and Their Condensed Products 8a–8d

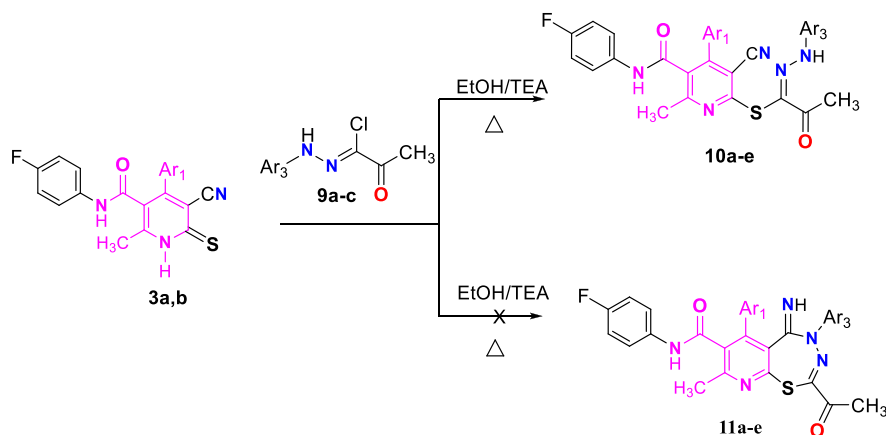


Compounds 7,8, a: X = CH₃, Ar₁ = 2,5-(CH₃O)₂-C₆H₃; b: X = CH₃, Ar₁ = 4-H₃C-C₆H₄;
c: X = F, Ar₁ = 2,5-(CH₃O)₂-C₆H₃; d: X = F, Ar₁ = 4-H₃C-C₆H₄

available in the market. For example, prasugrel,²¹ and ticlopidine and clopidogrel²² (Figure 2) were reported as antiplatelet drugs. Potent biological activities such as antileishmanial, antimicrobial, anti-inflammatory, anticancer, and antiplatelet agents were also observed from thienopyridine and their derivatives.^{23–29}

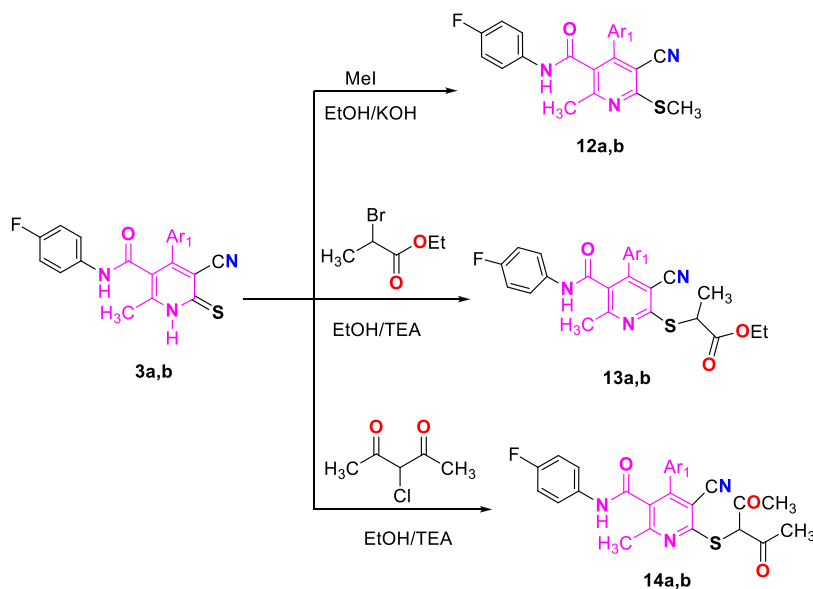
Prompted by these and as an extension of our preceding effort in the chemistry of pyridine, pyridinethione, and thienopyridine,^{30–33} we describe here the synthesis of a new series of substituted and condensed pyridines.

Scheme 4. Reaction of Pyridinethions 3a,b with Hydrazoyl Halides 9a–9c



Compounds **10**, a: $\text{Ar}_1=2,5\text{-(CH}_3\text{O)}_2\text{-C}_6\text{H}_3$, $\text{Ar}_3=\text{C}_6\text{H}_5$; b: $\text{Ar}_1=2,5\text{-(CH}_3\text{O)}_2\text{-C}_6\text{H}_3$, $\text{Ar}_3=4\text{-H}_3\text{C-C}_6\text{H}_4$; c: $\text{Ar}_1=4\text{-H}_3\text{C-C}_6\text{H}_4$, $\text{Ar}_3=4\text{-F-C}_6\text{H}_4$; d: $\text{Ar}_1=4\text{-H}_3\text{C-C}_6\text{H}_4$, $\text{Ar}_3=4\text{-H}_3\text{C-C}_6\text{H}_4$; e: $\text{Ar}_1=2,5\text{-(CH}_3\text{O)}_2\text{-C}_6\text{H}_3$, $\text{Ar}_3=4\text{-F-C}_6\text{H}_4$

Scheme 5. Reaction of Thienopyridines 3a,b with Halogenated Compounds



Compounds **3**, **12**, **13**, **14**, a: $\text{Ar}_1=2,5\text{-(CH}_3\text{O)}_2\text{-C}_6\text{H}_3$, b: $\text{Ar}_1=4\text{-CH}_3\text{-C}_6\text{H}_4$

2. RESULTS AND DISCUSSION

2.1. Chemistry. 3-Aryl-2-cyano-prop-2-enethioamide derivatives **1a,b** were refluxed with *N*-(4-fluorophenyl)-3-oxobutanamide **2** in ethanol, in the presence of a few drops of piperidine to give cyanopyridinethione derivatives **3a,b** (Scheme 1).

To explore the synthetic potentiality of pyridinethions **3a,b**, reactions with several reagents as ethyl chloroacetate, phenacyl chloride, chloroacetone, and chloroacetonitrile were performed, resulting in the formation of the corresponding *S*-alkylated derivatives **4a–4h**, respectively (Scheme 2).

Former compound **4a–4h** underwent intramolecular cyclization when refluxed in sodium ethoxide solution to provide an additional series of thieno[2,3-*b*]pyridine derivatives **5a–5h**, respectively. However, thieno[2,3-*b*]pyridine derivatives **5a–5h** could also be synthesized directly through the action of sodium ethoxide on pyridinethione **3** with α -halocarbonyl compounds. Structures of derivatives (**5a–5h**) were confirmed using both the elemental analysis plus spectral data.

When 2-thioxopyridine-3-carbonitrile **3a,b** was refluxed with derivatives of 2-chloro-*N*-arylacamide **6a,b** as 2-chloro-*N*-(4-fluorophenyl)acetamide or 2-chloro-*N*-(*p*-tolyl)acetamide³⁴ in EtOH containing a catalytic amount of Et_3N , the corresponding 2-(*N*-aryl)-carboxamidomethylthiopyridine derivatives **7a–7d** were obtained, respectively (Scheme 3).

Derivatives of thieno[2,3-*b*]pyridine-2,5-dicarboxamide **8a–8d** can be obtained by the cyclization of compounds **7a–7d** or directly from compounds **3a,b** with 2-chloro-*N*-arylacamide derivatives **6a,b**, in sodium ethoxide solution (Scheme 3). The structures of compound **8a–8d** were proved through elemental analysis in addition to spectral data. Where the ^1H NMR spectra for **8a–8d** derivatives were characterized by the absence of the CH_2 group singlet aforementioned in compounds **7a–7d** and the appearance of a peak near 6 ppm corresponding to NH_2 .

The study was concerned also in examining the activity of thiopyridine derivative **3** toward (*C*-acetyl)-*N*-arylhydrazonoyl chlorides **9a–9c** through refluxing them in ethanol containing

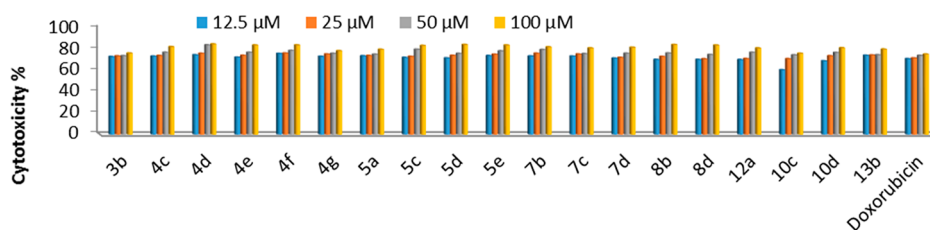


Figure 3. Dose dependent antiproliferative data of the 19 compounds against HCT-116 cancer cells according to the MTT assay after 48 h of exposure.

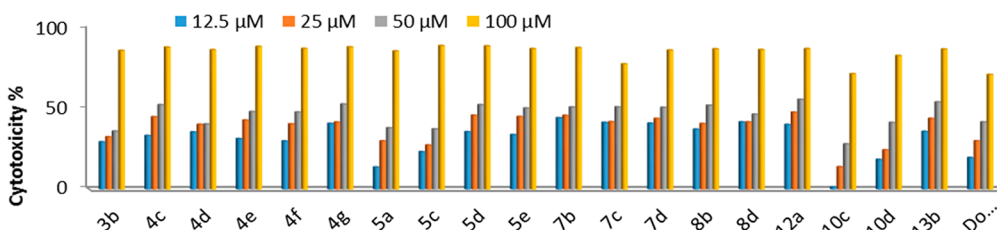


Figure 4. Dose dependent antiproliferative data of the 19 compounds against HepG2 cancer cells according to the MTT assay after 48 h of exposure.

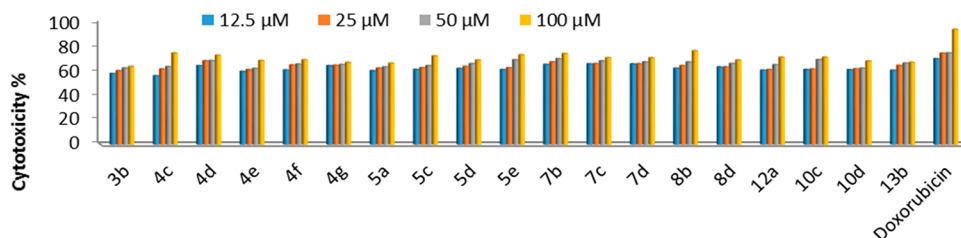


Figure 5. Dose dependent antiproliferative data of the 19 compounds against MCF-7 cancer cells according to the MTT assay after 48 h of exposure.

drops of trimethylamine, and the results showed that derivative **10** (open structure) and not derivative **11** (fused structure) was isolated from the reaction mixture (Scheme 4).

The structure of compounds **10a–10e** were determined by their elemental analysis and spectral data. Hence, the IR spectra showed bands owing to the CN group.

Finally, pyridinethione derivatives **3a,b** were reacted with various halogenated compounds such as (methyl iodide, 2-bromopropionate or 3-chloro-2,4-pentandione) and KOH or TEA under reflux to afford S-alkylated compounds **12**, **13**, and **14**, respectively (Scheme 5). The products structures **12a,b**, **13a,b**, and **14a,b** were established based on their correct spectral data and elemental analyses. In general, the IR spectra were characterized by one, two, and three bands assigned for the C=O group, respectively.

2.2. Antiproliferative Activity. The *in vitro* screening of a series of 19 derivatives for their activity against HCT-116, HepG-2, and MCF-7 human cancer cells and one human healthy cell line (BJ-1) were performed by the MTT assay. Intact cells percentages were calculated and compared with those of the control. Compounds activities against the three cell lines were compared to the activity of doxorubicin. A suppression in all cancer cells was observed in a dose-dependent manner (Figures 3 and 4). For HCT-116 human colorectal carcinoma cells, both Figure 3 and Table 4 show that 17 compounds (**5d**, **4d**, **4f**, **7b**, **5c**, **4e**, **4c**, **7c**, **8b**, **13b**, **4g**, **5a**, **7d**, **10d**, **8d**, **12a**, and **3b**, respectively) have more potent cytotoxic activities; two compounds (**10c** and **5e**, respectively) have significantly less cytotoxic activity against HCT-116 relative to that of doxorubicin. In the case of MCF-7 human breast cancer cells, two compounds (**7b** and **4c**, respectively) have slightly less cytotoxic activities; the rest of the compounds have essentially

less cytotoxic activities against MCF-7 relative to the reference drug (Figure 5 and Table 1). In the case of HepG2 human liver cancer cells, 18 compounds (**7b**, **12a**, **4g**, **5d**, **7d**, **13b**, **4c**, **8b**, **5e**, **8d**, **4e**, **7c**, **4f**, **4d**, **3b**, **5c**, **10d**, and **5a**, respectively) have strong activities; one compound (**10c**) has altogether less cytotoxic

Table 1. Antiproliferative IC₅₀ Values against the Three Cancer Cell Lines (MTT Assay)

compound code	IC ₅₀ (μM) ± SD		
	HCT-116	HepG-2	MCF-7
3b	19.7 ± 2.8	52.9 ± 5.1	38.7 ± 4.1
4c	15.4 ± 1.9	36.1 ± 3.9	32.8 ± 3.6
4d	10.3 ± 1.1	45.1 ± 4.6	24.8 ± 3.1
4e	15.2 ± 1.3	40.4 ± 4.1	34.7 ± 2.9
4f	12.3 ± 1.5	43.6 ± 4.3	30.9 ± 3.5
4g	17.2 ± 2.1	33.3 ± 3.1	30.8 ± 2.7
5a	17.3 ± 2.1	60.7 ± 5.8	34.5 ± 4.1
5c	14.8 ± 2.5	55.8 ± 5.2	30.6 ± 2.5
5d	9.5 ± 1.1	33.6 ± 3.7	30.8 ± 3.5
5e	26.7 ± 3.1	37.5 ± 3.8	28.3 ± 1.9
7b	13.7 ± 1.8	30.4 ± 3.2	23.2 ± 2.5
7c	15.9 ± 2.1	41.7 ± 4.1	26.3 ± 1.7
7d	17.5 ± 2.4	35.4 ± 4.1	26.5 ± 3.5
8b	16.3 ± 1.9	37.4 ± 3.9	26.3 ± 3.1
8d	18.1 ± 2.6	37.8 ± 4.3	30.4 ± 3.7
12a	18.4 ± 3.1	31.6 ± 3.1	31.9 ± 2.9
10c	25.1 ± 3.5	85.1 ± 5.9	29.6 ± 2.5
10d	17.8 ± 2.5	59.3 ± 5.1	34.3 ± 3.5
13b	17.1 ± 2.4	35.9 ± 3.5	31.8 ± 2.8
doxorubicin	21.8 ± 2.9	63.2 ± 5.8	16.7 ± 1.5

Table 2. Docking results of 4d, 4f, 5c, 5d and 7b with receptor of 4k9g

cmpd	ligand moiety	receptor site	interacting residues (type of interaction)	distance (Å)	E (kcal/mol)	docking score (kcal/mol)
4d						-9.3347
4f	S 1	NE2 HIS 62 (B)	H-acceptor	3.33	-1.2	-8.2076
	O 45	NE2 HIS 62 (A)	H-acceptor	3.06	-3.1	
5c						-8.0733
5d	O 54	ND2 ASN 97 (A)	H-acceptor	3.27	-2.2	-9.4430
7b	O 37	NE2 HIS 62 (C)	H-acceptor	2.76	-6.0	-8.8233

Table 3. Docking Results of 4c, 5d, 4g, 7b, 7d, 12a, and 13b with the Receptor of 4dk7

cmpd	ligand moiety	receptor site	interacting residues (type of interaction)	distance (Å)	E (kcal/mol)	docking score (kcal/mol)
4c	S 1	O ALA 359 (A)	H-donor	3.91	-0.7	-5.9269
	6-ring	CB MSE 423 (A)	π -H	4.20	-1.0	
5d	S 15	OE1 GLU 355 (A)	H-donor	3.56	-2.2	-4.5118
4g						-6.3130
7b	6-ring	OH TYR 97 (A)	π -H	4.70	-0.8	-7.6102
7d	O 40	NZ LYS 91 (A)	H-acceptor	2.87	-3.0	-7.6825
	N 58	CA VAL 87 (A)	H-acceptor	3.18	-1.2	
	6-ring	CB PRO 82 (A)	π -H	4.21	-0.6	
12a						-7.2737
13b						-7.2510

Table 4. Docking Results of 4d, 5e, 7c, 7d, 8b, and 10c with the Receptor 6p05

cmpd	ligand moiety	receptor site	interacting residues (type of interaction)	distance (Å)	E (kcal/mol)	docking score (kcal/mol)
4d						-7.3447
5e						-7.5894
7c						-7.1441
7d						-7.5635
8b	6-ring	CD1 LEU 92 (A)	π -H	4.00	-0.6	-6.7931
	6-ring	OH TYR 97 (A)	π -H	4.45	-0.8	
10c						-7.5544

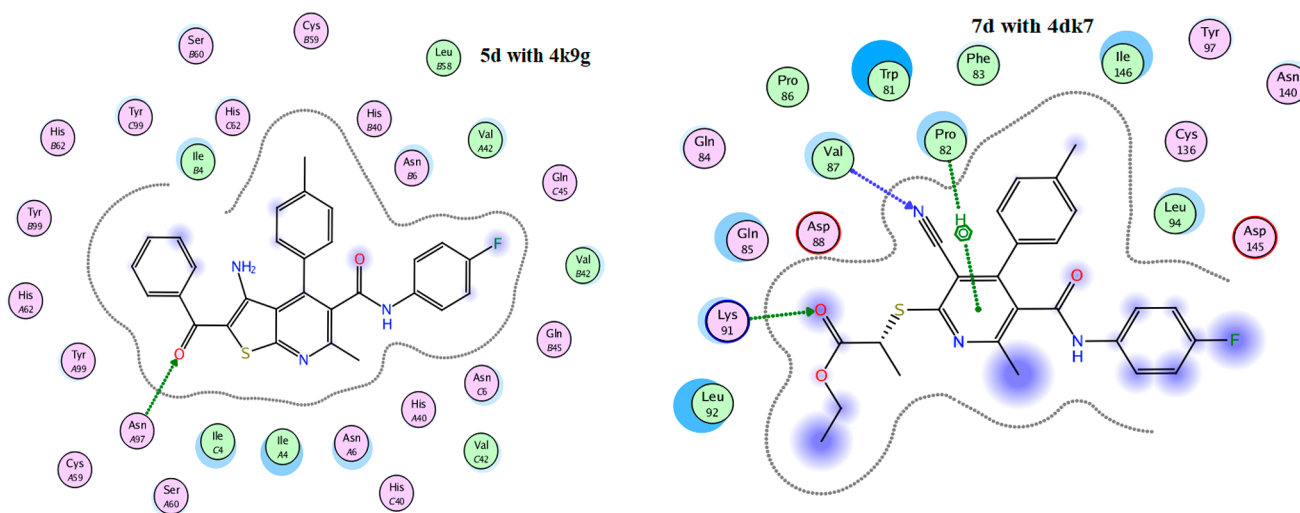


Figure 6. 2D docked model of compound 5d and 7d, respectively, into the active site of 4k9g and 4dk7.

activity against HepG2 relative to that of doxorubicin (Figure 4 and Table 1). From the above results, it is possible to conclude that compounds 3b, 4c–5d, 7b–12a, 10d, 13b are selectively active on both human liver and colon cancer types but not active on the human breast cancer type. Compound 5e is specifically active on only the human liver cancer type but not active on both the human colon and breast cancer types. Compound 10c is selectively active on only human colon cancer but not active on

both the human liver and breast cancer types. All compounds were tested against the nontumor fibroblast-derived cells line (BJ-1) and demonstrated very low cytotoxicity.

2.3. Molecular Docking. Molecular docking has become a widespread tool for drug discovery.^{35,36} To illustrate the potential binding mode of pyridine derivatives into the active sites of the three types of tumor cells we investigated (HCT-116, HepG-2 and MCF-7), a docking study of the most active

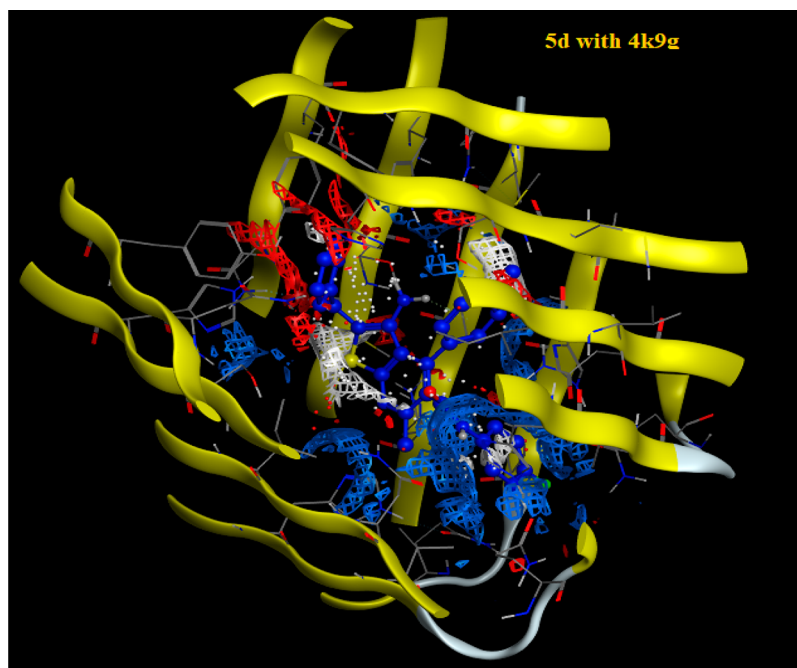


Figure 7. Electrostatic map of **5d** on the active site of **4k9g**.

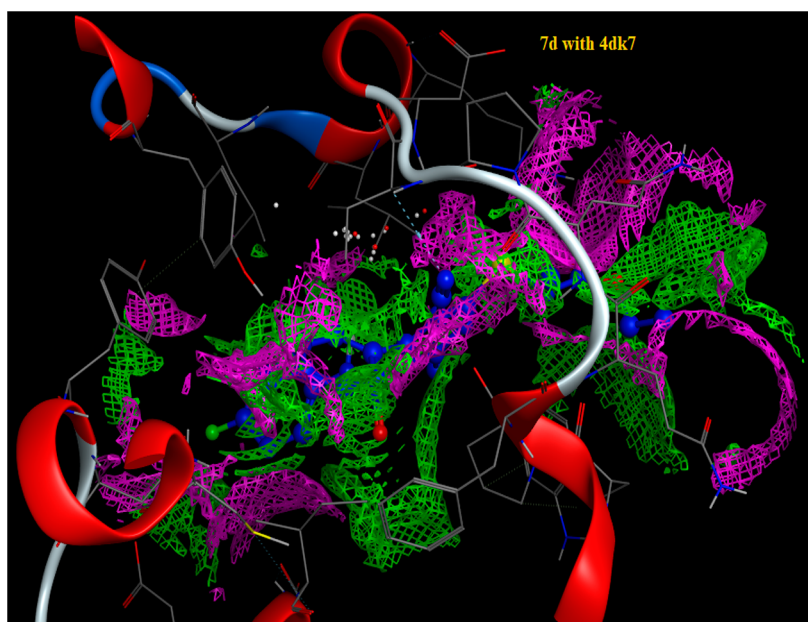


Figure 8. Contact performance of **7d** on the active site of **4dk7**.

derivatives against each cell type using MOE (2014.0901) (Molecular Operating Environment software). The molecular docking studies were performed using (4k9g), (4dk7), and (6p05), the cocrystal structures of proteins for colon, human liver, and breast cancer cells, respectively. The results are presented in Tables 2–4 and Figures 6–9. From the results of docking study, we noticed that the highest binding interactions were found between the pyridine derivatives **4d**, **4f**, **5c**, **5d**, and **7b** with 4k9g of colon cancer (−9.4430 to −8.0733 kcal/mol) which is compatible with experimental results of the highest activity against colon cancer HCT-116 (Table 4). The most reactive derivative **5d** showed H-acceptor interaction toward the 4k9g protein with fit docking paths (bond length ≤ 3.5 Å) (Figures 6 and 7). In the case of 4dk7 for human liver, all tested

pyridine derivatives showed interactions with docking scores ranging from −7.6825 to −4.5118 (Table 3). The two more reactive pyridine derivatives **7b** and **7d** showed a π -H interaction of the pyridine ring toward TYR97 and PRO82 amino acid residues. The pyridine derivative **7d** showed two other H-acceptor interactions with the amino acid residues VAL87 and LYS91 due to the presence of C \equiv N and C=O groups (Figure 4). The H-acceptor interaction with HIS-62 is the common type of interaction in derivatives **4f** and **7b** (Table 4 and Figure 8). The pyridine derivatives exert low anticancer activity on the MCF-7 cell line. This is consistent with the data shown in Table 4 as all derivatives did not interact with the amino acids of 6p05 protein, except derivative **8b** (Figure 9).

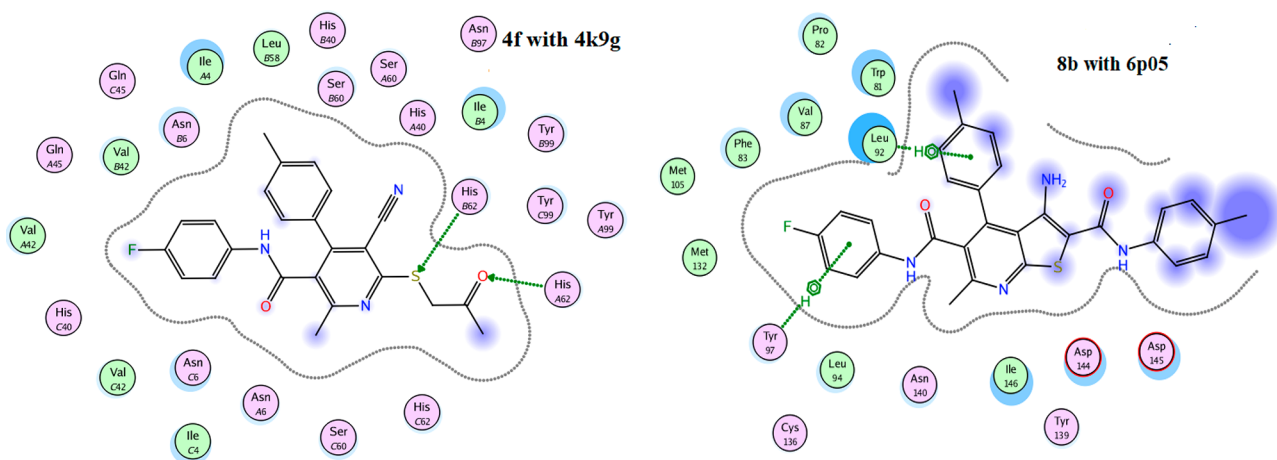


Figure 9. 2D docked model of compounds **4f** and **8b**, respectively, into the active site of 4k9g and 6p05.

The latter incurred π -H interaction of the 6-ring with LEU92 and TYR97 amino acids.

3. STRUCTURE ACTIVITY RELATIONSHIPS

By correlation of the afforded activity results against the tested cancer cell lines with the characteristic structural features (SAR) of the most active compounds, taking into account the docking results, the effect of aromatic substitution in hydrazonyl chlorides, **6a,b** and also (C-acetyl)-*N*-arylhydrazonylchlorides **9a–9c** on the activities of the synthesized compounds **7a–7d** and **10a–10e** is more evident. Thus, in the light of our current results (Figures 3–5), we can deduce that the presence of the methyl group or fluoro atom on the aromatic rings lead to an increased inhibitory behavior. It is conspicuous that the efficiency was minimized in other compounds which do not include such structural functional moieties in their structural skeleton.

The structures are usually diverse from what we have seen in comparative structures in which the methyl group and fluoro atom are linked to aromatic ring in the pyridine ring system. Within the current work, the foremost successful structures were the substituted pyridine derivatives **7b**, **7c**, **7d**, and **10d** when tested considering doxorubicin as a standard drug. The contrast in action between the newly synthesized compounds may be credited to the shown connections of the molecule's pyridine ring.

4. CONCLUSIONS

This report presents a convenient and efficient synthesis of novel pyridinethione and thienopyridine derivatives. The new products were screened for their cytotoxic activity against human cancer cell lines. Several compounds were selectively active on both human liver and colon cancer cells but not active on human breast cancer cells. All compounds demonstrated low cytotoxicity when screened against a nontumor fibroblast-derived cell line thereby providing a good safety profile as potential anticancer agents. Molecular docking analysis illustrated that the synthesized compounds are predicted to fit into the binding sites of the target 4k9g and 4dk7 proteins associated with specific cancer cell types.

5. EXPERIMENTAL SECTION

5.1. Synthesis. All melting points were measured on a Gallenkamp Melting point apparatus and are uncorrected. The

IR spectra were recorded on a Shimadzu FT-IR 8101 PC infrared spectrophotometer (Shimadzu, Tokyo, Japan) using KBr disks. The NMR spectra were preserved on a Varian Mercury VX-400 NMR spectrometer (Varian, Palo Alto, CA). ^1H NMR spectra were run at 400 MHz and ^{13}C NMR spectra were run at 100 MHz in deuterated chloroform (CDCl_3) or dimethyl sulfoxide ($\text{DMSO}-d_6$) as specified in individual compound characterizations. Chemical shifts are given in parts per million and were referenced to those of the solvents. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer at 70 eV. Elemental analyses were registered on an Elementar-Vario EL (Germany) automatic analyzer. The molecular docking studies have been performed using MOE (2014.0901) (Molecular Operating Environment software). The molecular docking studies were obtained using (4k9g), (4dk7), and (6p05), the cocrystal structures of proteins for colon, human liver, and breast cancer cells, respectively, using MOE (2014.0901) (Molecular Operating Environment software).

5.1.1. Preparation of Pyridine-2(1H) Thione Derivatives (3a, 3b). In absolute ethanol (15 mL), a mixture of the appropriate 3-aryl-2-cyano-prop-2-enethioamide derivative **1a** or **1b** (10 mmol), *N*-(4-fluorophenyl)-3-oxobutanamide **2** (10 mmol), and a few drops of piperidine were heated under reflux for 5–8 h. The reaction mixture was then allowed to cool before the precipitate was filtered, dried, and crystallized from ethanol to get the pyridine-2(1H) thione derivatives.

5.1.1.1. 5-Cyano-4-(2,5-dimethoxyphenyl)-*N*-(4-fluorophenyl)-6-mercapto-2-methylnicotinamidemethylnicotinamide (3a). Orange solid (81%); mp: 170–172 °C (EtOH); IR (KBr, ν , cm^{-1}): 3454 (NH), 3252 (NH), 2229 (CN), 1644 (C=O); ^1H NMR ($\text{DMSO}-d_6$): δ 2.51 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 7.13–8.32 (m, 7H, CH-Ar), 9.60 (bs, 1H, NH), 10.10 (bs, 1H, NH); ^{13}C NMR ($\text{DMSO}-d_6$): δ 23.21, 56.03, 56.81, 113.11, 113.37, 113.56, 116.79, 120.36, 121.32, 121.82, 121.87, 143.12, 153.16, 153.46, 192.65; MS m/z (%): 423 [M^+] (95). Anal. calcd for $\text{C}_{22}\text{H}_{18}\text{FN}_3\text{O}_3\text{S}$: C, 62.40; H, 4.28; N, 9.92. Found: C, 62.42; H, 4.25; N, 9.90.

5.1.1.2. 5-Cyano-*N*-(4-fluorophenyl)-2-methyl-6-thioxo-4-(*p*-tolyl)-1,6-dihydropyridine-3-carboxamide (3b). Yellow solid (86%); mp: 228–230 °C (EtOH); IR (KBr, ν , cm^{-1}): 3448 (NH), 3221 (NH), 2222 (CN), 1650 (C=O); ^1H NMR ($\text{DMSO}-d_6$): δ 2.43 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 6.95–7.89 (m, 8H, CH-Ar), 9.65 (bs, 1H, NH), 10.42 (bs, 1H, NH); MS

m/z (%): 377 [M^+] (29.55), 267 (100), 110 (13), 359 (16.38), 82 (33). Anal. calcd for $C_{21}H_{16}FN_3OS$: C, 66.83; H, 4.27; N, 11.13. Found: C, 66.85; H, 4.26; N, 11.15.

5.1.2. Preparation of Compounds 4a–4h. **5.1.2.1. Method A.** A few drops of triethylamine were added to a mixture of **3a** or **3b** (10 mmol) and the appropriate α -haloketone (namely, ethyl chloroacetate, phenacyl chloride, chloroacetone, or chloroacetonitrile) (10 mmol) in absolute ethanol (15 mL). The reaction mixture was allowed to cool after being refluxed for 3–5 h. Compounds **4a–4h** were obtained by filtering off the produced precipitate, drying, and crystallizing it from the proper solvent.

5.1.2.2. Method B. A white to pale yellow precipitate was noticed after stirring a mixture of **3a** or **3b** (10 mmol), the appropriate α -haloketone (namely, ethyl chloroacetate, phenacyl chloride, chloroacetone, or chloroacetonitrile) (10 mmol) and potassium hydroxide (10 mmol) in acetone (15 mL) for 10 h. Compounds **4a–4h** were obtained by filtering the generated precipitate, drying, and crystallizing it from ethanol.

5.1.2.3. Ethyl-2-((3-cyano-4-(2,5-dimethoxyphenyl)-5-(4-fluorophenyl)-carbamoyl)-6-methylpyridin-2-yl)thio)acetate (4a). White solid [A] (90%), [B] (65%); mp: 201–204 °C (EtOH); IR (KBr, ν , cm^{-1}): 3240 (NH), 2221 (CN), 1747 (C=O), 1636 (C=O); 1H NMR (DMSO- d_6): δ 1.26 (t, 3H, CH_3), 2.55 (s, 3H, CH_3), 3.70 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 4.17 (s, 2H, CH_2), 4.19 (q, 2H, CH_2), 6.95–7.46 (m, 7H, CH-Ar), 10.52 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 14.56, 23.28, 33.05, 55.27, 56.32, 61.55, 104.65, 113.41, 115.47, 116.13, 116.51, 121.72, 123.82, 130.08, 134.94, 148.89, 150.23, 153.06, 158.57, 159.31, 160.66, 163.80, 169.02; MS m/z (%): 509 [M^+] (45.32), 478 (12.15), 399 (100), 311 (15.40). Anal. calcd for $C_{26}H_{24}FN_3O_5S$: C, 61.29; H, 4.75; N, 8.25. Found: C, 61.31; H, 4.77; N, 8.22.

5.1.2.4. Ethyl-2-((3-cyano-5-(4-fluorophenyl)carbamoyl)-6-methyl-4-(*p*-tolyl)pyridin-2-yl)thio)acetate (4b). Yellow solid [A] (93%), [B] (70%); mp: 166–168 °C (EtOH); IR (KBr, ν , cm^{-1}): 3232 (NH), 2229 (CN), 1741 (C=O), 1642 (C=O); 1H NMR (DMSO- d_6): δ 1.20 (t, 3H, CH_3), 2.31 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 4.13 (s, 2H, CH_2), 4.15 (q, 2H, CH_2), 7.07–7.38 (m, 8H, CH-Ar), 10.43 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 14.10, 21.20, 21.70, 31.90, 61.10, 105.30, 113.30, 116.20, 123.10, 126.50, 127.40, 130.20, 132.60, 134.70, 155.10, 162.50, 163.20, 164.80, 168.20, 170.10; MS m/z (%): 463 [M^+] (25), 353 (15), 307 (36), 110 (85), 83(100). Anal. calcd for $C_{25}H_{22}FN_3O_5S$: C, 64.78; H, 4.78; N, 9.07. Found: C, 64.80; H, 4.77; N, 9.10.

5.1.2.5. 5-Cyano-4-(2,5-dimethoxyphenyl)-*N*-(4-fluorophenyl)-2-methyl-6-((2-oxo-2-phenylethyl)thio)nicotinamide (4c). Pale yellow solid [A] (90%); [B] (72%); mp: 193–195 °C (EtOH). IR (KBr, ν , cm^{-1}): 3272 (NH), 2225 (CN), 1696 (C=O), 1655 (C=O); 1H NMR (DMSO- d_6): δ 2.51 (s, 3H, CH_3), 3.63 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 4.92 (s, 2H, CH_2), 6.93–8.14 (m, 12H, CH-Ar), 10.47 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 22.60, 37.53, 56.33, 56.99, 105.02, 113.75, 115.86, 116.89, 121.73, 123.15, 129.04, 129.72, 134.28, 135.30, 136.64, 149.19, 150.60, 153.72, 158.27, 159.62, 161.42, 164.17, 193.43; MS m/z (%): 541 [M^+] (6), 510 (16), 105 (100), 77 (62). Anal. calcd for $C_{30}H_{24}FN_4O_5S$: C, 66.53; H, 4.47; N, 7.76. Found: C, 66.55; H, 4.49; N, 7.73.

5.1.2.6. 5-Cyano-*N*-(4-fluorophenyl)-2-methyl-6-((2-oxo-2-phenylethyl)thio)-4-(*p*-tolyl)-nicotinamide (4d). White solid [A] (89%); [B] (73%); mp: 205–207 °C (EtOH); IR (KBr, ν , cm^{-1}): 3434 (NH), 2222 (CN), 1705 (C=O), 1656 (C=O); 1H NMR (DMSO- d_6): δ 2.25 (s, 3H, CH_3), 2.32 (s,

3H, CH_3), 4.95 (s, 2H, CH_2), 7.09–8.14 (m, 13H, CH-Ar), 10.46 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 21.31, 22.86, 39.73, 103.95, 115.45, 115.84, 115.97, 121.87, 121.91, 128.70, 128.74, 129.31, 129.53, 131.51, 133.99, 136.87, 139.89, 151.77, 158.29, 161.29, 164.12, 193.84; MS m/z (%): 495 [M^+] (65), 463 (5), 384 (100), 105 (55). Anal. calcd for $C_{29}H_{22}FN_3O_5S$: C, 70.29; H, 4.47; N, 8.48. Found: C, 70.31; H, 4.44; N, 8.46.

5.1.2.7. 5-Cyano-4-(2,5-dimethoxyphenyl)-*N*-(4-fluorophenyl)-2-methyl-6-((2-oxopropyl)thio)nicotinamide (4e). Yellow solid [A] (88%); [B] (75%); mp: 187–190 °C (EtOH); IR (KBr, ν , cm^{-1}): 3272 (NH), 2220 (CN), 1668 (C=O), 1649 (C=O); 1H NMR (DMSO- d_6): δ 2.37 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 4.62 (s, 2H, CH_2), 6.95–7.45 (m, 7H, CH-Ar), 10.78 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 23.29, 29.55, 39.79, 55.65, 56.32, 105.69, 112.33, 114.79, 115.88, 116.13, 123.15, 130.08, 134.94, 149.15, 150.22, 153.07, 158.24, 158.94, 160.65, 163.79, 202.06; MS m/z (%): 479 [M^+] (47), 448 (69), 369 (100), 82 (35). Anal. calcd for $C_{25}H_{22}FN_3O_4S$: C, 62.62; H, 4.62; N, 8.76. Found: C, 62.64; H, 4.59; N, 8.77.

5.1.2.8. 5-Cyano-*N*-(4-fluorophenyl)-2-methyl-6-((2-oxopropyl)thio)-4-(*p*-tolyl)nicotinamide (4f). White solid [A] (91%); [B] (78%); mp: 198–200 °C (EtOH). IR (KBr, ν , cm^{-1}): 3235 (NH), 2215 (CN), 1736 (C=O), 1644 (C=O); 1H NMR (DMSO- d_6): δ 2.32 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 4.28 (s, 2H, CH_2), 7.13–7.41 (m, 8H, CH-Ar), 10.49 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 21.31, 23.19, 29.76, 39.98, 104.04, 115.40, 115.87, 116.00, 121.87, 128.74, 129.33, 129.52, 131.52, 134.95, 139.89, 151.78, 158.30, 158.45, 161.39, 201.97; MS m/z (%): 433 [M^+] (30), 390 (25), 323 (100), 281 (83), 110 (44). Anal. calcd for $C_{24}H_{20}FN_3O_2S$: C, 66.50; H, 4.65; N, 9.69. Found: C, 66.53; H, 4.62; N, 9.67.

5.1.2.9. 5-Cyano-6-((cyanomethyl)thio)-4-(2,5-dimethoxyphenyl)-*N*-(4-fluorophenyl)-2-methylthio)nicotinamide (4g). Yellow solid [A] (89%); [B] (73%); mp: 140–144 °C (EtOH); IR (KBr, ν , cm^{-1}): 3451 (NH), 2229 (CN), 1684 (C=O); 1H NMR (DMSO- d_6): δ 2.68 (s, 3H, CH_3), 3.64 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 4.44 (s, 2H, CH_2), 6.98–7.49 (m, 7H, CH-Ar), 10.59 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 19.31, 22.98, 55.43, 56.51, 105.78, 113.08, 115.40, 116.36, 117.41, 118.37, 122.33, 123.33, 123.67, 130.98, 134.93, 149.86, 150.51, 154.10, 158.43, 159.48, 164.09; MS m/z (%): 462 [M^+] (98), 352 (90), 69 (100). Anal. calcd for $C_{24}H_{19}FN_4O_3S$: C, 62.33; H, 4.14; N, 12.11. Found: C, 62.30; H, 4.12; N, 12.15.

5.1.2.10. 5-Cyano-6-((cyanomethyl)thio)-*N*-(4-fluorophenyl)-2-methyl-4-(*p*-tolyl)nicotinamide (4h). Yellow solid [A] (87%); [B] (71%); mp: 230–232 °C (EtOH); IR (KBr, ν , cm^{-1}): 3403 (NH), 2226 (CN), 1649 (C=O); 1H NMR (DMSO- d_6): δ 2.26 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 4.39 (s, 2H, CH_2), 7.02–7.46 (m, 8H, CH-Ar), 10.52 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 17.30, 21.10, 21.80, 114.60, 115.80, 117.20, 124.30, 127.60, 128.10, 131.20, 132.70, 135.10, 154.80, 162.70, 163.20, 164.20, 169.30, 171.30; MS m/z (%): 416 [M^+] (5); Anal. calcd for $C_{23}H_{17}FN_4OS$: C, 66.33; H, 4.11; N, 13.45. Found: C, 66.36; H, 4.09; N, 13.42.

5.1.3. Preparation of Compounds 5a–5h. **5.1.3.1. Method A.** Compounds **4a–4h** (10 mmol) were refluxed for 3–6 h in sodium ethoxide solution (0.23 g Na in 100 mL of EtOH). After cooling the mixture, a white to pale yellow solid precipitated in each case, which was then filtered out, dried, and crystallized from the specified solvent to provide the thieno[2,3-*b*]pyridine derivatives **5a–5h**.

5.1.3.2. Method B. For 3 h, a mixture of compound **3** (10 mmol) and the appropriate α -haloketone (10 mmol) (namely, ethyl chloroacetate, phenacyl chloride, chloroacetone, or chloroacetonitrile) was refluxed in sodium ethoxide solution (0.23 g Na in 100 mL of EtOH). The produced precipitate was then filtered out, dried, and crystallized from the specified solvent, yielding the thieno[2,3-*b*]pyridine derivatives (**5a–5h**).

5.1.3.3. Ethyl-3-amino-4-(2,5-dimethoxyphenyl)-5-((4-fluorophenyl)-carbamoyl)-6-methyl-thieno[2,3-*b*]pyridine-2-carboxylate (5a**).** White solid [A] (89%); [B] (75%); mp: 175–178 °C (EtOH); IR (KBr, ν , cm^{-1}): 3481–3311 (NH_2), 3260 (NH), 1730 (C=O), 1674 (C=O); ^1H NMR (DMSO- d_6): δ 1.30 (t, 3H, CH_3), 2.55 (s, 3H, CH_3), 3.65 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 4.24 (q, 2H, CH_2), 6.90–7.44 (m, 9H, 7CH-Ar + NH_2), 10.37 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 13.89, 23.65, 55.65, 56.69, 60.12, 104.65, 113.38, 115.48, 116.89, 120.02, 121.36, 123.15, 129.71, 131.13, 135.31, 140.83, 148.86, 150.58, 152.69, 155.82, 157.51, 158.57, 159.33, 164.85, 207.02; MS m/z (%): 509 [M^+] (23), 437 (8), 399 (25), 110 (65), 83 (100). Anal. calcd for $\text{C}_{26}\text{H}_{24}\text{FN}_3\text{O}_5\text{S}$: C, 61.29; H, 4.75; N, 8.25. Found: C, 61.31; H, 4.72; N, 8.29.

5.1.3.4. Ethyl-3-amino-5-((4-fluorophenyl)carbamoyl)-6-methyl-4-(*p*-tolyl)thieno[2,3-*b*]pyridine-2-carboxylate (5b**).** Yellow solid; [A] (92%); [B] (70%); mp: 250–252 °C (EtOH); IR (KBr, ν , cm^{-1}): 3446–3311 (NH_2), 3257 (NH), 1710 (C=O), 1647 (C=O); ^1H NMR (DMSO- d_6): δ 1.03 (t, 3H, CH_3), 2.31 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 4.37 (q, 2H, CH_2), 5.23 (bs, 2H, NH_2), 6.90–7.44 (m, 8H, CH-Ar), 10.40 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 15.10, 20.80, 21.40, 61.33, 116.40, 119.30, 123.60, 126.10, 126.70, 127.40, 130.20, 131.20, 132.20, 135.40, 136.30, 157.10, 161.50, 163.60, 164.80, 165.30, 168.40; MS m/z (%): 463 [M^+] (2), 391 (35), 280 (100), 82 (63). Anal. calcd for $\text{C}_{25}\text{H}_{22}\text{FN}_3\text{O}_3\text{S}$: C, 64.78; H, 4.78; N, 9.07. Found: C, 64.74; H, 4.80; N, 9.11.

5.1.3.5. 3-Amino-2-benzoyl-4-(2,5-dimethoxyphenyl)-*N*-(4-fluorophenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxamide (5c**).** Yellow solid [A] (86%); [B] (73%); mp: 165–168 °C (EtOH); IR (KBr, ν , cm^{-1}): 3465–3314 (NH_2), 3280 (NH), 1721 (C=O), 1656 (C=O); ^1H NMR (DMSO- d_6): δ 2.51 (s, 3H, CH_3), 3.66 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 7.03–7.79 (m, 14H, 12CH-Ar + NH_2), 10.39 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 23.21, 56.03, 56.70, 103.49, 113.75, 115.82, 115.95, 117.12, 120.16, 121.76, 121.81, 122.44, 127.76, 129.03, 131.10, 131.74, 141.16, 142.11, 150.43, 151.00, 153.31, 157.47, 158.17, 161.14, 164.93, 189.46; MS m/z (%): 541 [M^+] (90). Anal. calcd for $\text{C}_{30}\text{H}_{24}\text{FN}_4\text{O}_3\text{S}$: C, 66.53; H, 4.47; N, 7.76. Found: C, 66.55; H, 4.48; N, 7.73.

5.1.3.6. 3-Amino-2-benzoyl-*N*-(4-fluorophenyl)-6-methyl-4-(*p*-tolyl)thieno[2,3-*b*]pyridine-5-carboxamide (5d**).** Pale yellow solid [A] (91%); [B] (72%); mp: 284–286 °C (EtOH); IR (KBr, ν , cm^{-1}): 3471–3283 (NH_2), 3147 (NH), 1699 (C=O), 1644 (C=O); ^1H NMR (DMSO- d_6): δ 2.36 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 7.11–7.78 (m, 15H, 13CH-Ar + NH_2), 10.47 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 21.41, 23.14, 103.58, 115.80, 115.93, 119.57, 121.91, 121.95, 127.78, 128.72, 129.03, 129.76, 130.56, 130.93, 131.75, 139.45, 141.13, 145.16, 150.82, 157.28, 158.23, 159.60, 161.35, 165.06, 189.49; MS m/z (%): 495 [M^+] (9), 350 (18), 216 (100), 111 (42), 77 (82). Anal. calcd for $\text{C}_{29}\text{H}_{22}\text{FN}_3\text{O}_2\text{S}$: C, 70.29; H, 4.47; N, 8.48. Found: C, 70.27; H, 4.44; N, 8.51.

5.1.3.7. 2-Acetyl-3-amino-4-(2,5-dimethoxyphenyl)-*N*-(4-fluorophenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxamide (5e**).** Yellow solid [A] (88%); [B] (73%); mp: 200–202

°C (EtOH); IR (KBr, ν , cm^{-1}): 3467–3297 (NH_2), 3142 (NH), 1674 (C=O), 1652 (C=O); ^1H NMR (DMSO- d_6): δ 2.37 (s, 3H, CH_3), 2.65 (s, 3H, CH_3), 3.63 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 6.99–7.46 (m, 9H, CH-Ar + NH_2), 10.38 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 23.14, 29.55, 56.00, 56.65, 105.08, 115.80, 115.93, 116.96, 120.59, 121.74, 121.78, 122.48, 131.03, 135.21, 141.88, 148.36, 150.45, 153.21, 157.02, 158.15, 159.52, 159.71, 164.99, 192.16; MS m/z (%): 479 [M^+] (41), 248 (25), 217 (100), 60 (93). Anal. calcd for $\text{C}_{25}\text{H}_{22}\text{FN}_3\text{O}_4\text{S}$: C, 62.62; H, 4.62; N, 8.76. Found: C, 62.64; H, 4.59; N, 8.77.

5.1.3.8. 2-Acetyl-3-amino-*N*-(4-fluorophenyl)-6-methyl-4-(*p*-tolyl)thieno[2,3-*b*]pyridine-5-carboxamide (5f**).** Yellow solid [A] (92%); [B] (71%); mp: 250–253 °C (EtOH); IR (KBr, ν , cm^{-1}): 3473–3387 (NH_2), 3304 (NH), 1659 (C=O), 1619 (C=O); ^1H NMR (DMSO- d_6): δ 2.30 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 2.60 (s, 3H, CH_3), 6.36–7.40 (m, 10H, CH-Ar + NH_2), 10.45 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 20.60, 21.60, 53.10, 115.40, 119.30, 123.20, 126.10, 126.80, 127.50, 130.40, 131.20, 132.20, 135.20, 136.40, 157.30, 160.70, 163.40, 164.90, 165.50, 168.10; MS m/z (%): 433 [M^+] (7), 323 (9), 110 (98), 83 (100). Anal. calcd for $\text{C}_{24}\text{H}_{20}\text{FN}_3\text{O}_2\text{S}$: C, 66.50; H, 4.65; N, 9.69. Found: C, 66.52; H, 4.62; N, 9.72.

5.1.3.9. 3-Amino-2-cyano-4-(2,5-dimethoxyphenyl)-*N*-(4-fluorophenyl)-6-methylthieno[2,3-*b*]pyridine-5-carboxamide (5g**).** Yellow solid [A] (89%); [B] (72%); mp: 190–194 °C. IR (KBr, ν , cm^{-1}): 3450–3263 (NH_2), 3148 (NH), 2228 (CN), 1647 (C=O); ^1H NMR (DMSO- d_6): δ 2.66 (s, 3H, CH_3), 3.68 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 6.88–7.48 (m, 9H, 7CH-Ar + NH_2), 10.54 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ : 23.01, 55.46, 56.04, 105.30, 112.96, 114.57, 115.19, 115.64, 116.28, 116.53, 117.50, 121.18, 121.37, 123.50, 126.36, 130.32, 149.03, 150.71, 152.21, 156.85, 158.66, 161.82, 163.18, 177.57 MS m/z (%): 462 [M^+] (12), 423 (15), 352 (50), 118 (100). Anal. calcd for $\text{C}_{24}\text{H}_{19}\text{FN}_4\text{O}_3\text{S}$: C, 62.33; H, 4.14; N, 12.11. Found: C, 62.30; H, 4.12; N, 12.14.

5.1.3.10. 3-Amino-2-cyano-*N*-(4-fluorophenyl)-6-methyl-4-(*p*-tolyl)thieno[2,3-*b*]pyridine-5-carboxamide (5h**).** Yellow solid [A] (93%); [B] (74%); mp: 183–185 °C. IR (KBr, ν , cm^{-1}): 3396–3209 (NH_2), 3139 (NH), 2228 (CN), 1653 (C=O); ^1H NMR (DMSO- d_6): δ 2.33 (s, 3H, CH_3), 2.53 (s, 3H, CH_3), 7.15–7.36 (m, 10H, 8CH-Ar + NH_2), 10.54 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ : 20.30, 21.40, 114.10, 115.50, 119.40, 123.60, 126.30, 126.60, 127.50, 130.60, 131.40, 132.30, 135.30, 136.50, 157.40, 163.50, 164.80, 165.60, 167.10; MS m/z (%): 416 [M^+] (8). Anal. calcd for $\text{C}_{23}\text{H}_{17}\text{FN}_4\text{O}_3\text{S}$: C, 66.33; H, 4.11; N, 13.45. Found: C, 66.35; H, 4.08; N, 13.43.

5.1.4. Preparation of Compounds **7a–7d.** A few drops of triethylamine were added to a mixture of compounds **3a** or **3b** (10 mmol) and 2-chloro-*N*-arylacetyl derivatives **6a** or **6b** (10 mmol) in absolute ethanol (15 mL), and the mixture was refluxed for 2 h. The reaction was allowed to cool before the precipitate was filtered, dried, and crystallized from ethanol to produce the compounds **7a–7d**.

5.1.4.1. 5-Cyano-4-(2,5-dimethoxyphenyl)-*N*-(4-fluorophenyl)-2-methyl-6-((2-oxo-2-(*p*-tolylamino)ethyl)thio)nicotinamide (7a**).** Yellow solid (82%); mp: 268–270 °C (EtOH); IR (KBr, ν , cm^{-1}): 3468 (NH), 3328 (NH), 2221 (CN), 1675 (C=O), 1627 (C=O); ^1H NMR (DMSO- d_6): δ 2.34 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 3.68 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 4.17 (s, 2H, CH_2), 6.95–7.87 (m, 11H, CH-Ar), 9.50 (bs, 1H, NH), 10.45 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 20.90, 21.50, 40.10, 56.80, 57.10, 105.20, 112.60, 113.50, 113.90, 115.90, 116.10, 122.50, 123.10, 127.30, 129.20, 129.60,

134.60, 135.10, 136.80, 150.40, 154.30, 155.60, 162.30, 163.10, 165.30, 168.60, 170.20; MS m/z (%): 570 [M^+] (40). Anal. calcd for $C_{31}H_{27}FN_4O_4S$: C, 65.25; H, 4.77; N, 9.82. Found: C, 65.28; H, 4.79; N, 9.80.

5.1.4.2. 5-Cyano-*N*-(4-fluorophenyl)-2-methyl-6-((2-oxo-2-(*p*-tolylamino)ethyl)-thio)-4-(*p*-tolyl)nicotinamide (7b). White solid (81%); mp: 235–237 °C (EtOH); IR (KBr, ν , cm^{-1}): 3271 (NH), 3141 (NH), 2220 (CN), 1667 (C=O), 1649 (C=O); 1H NMR (DMSO- d_6): δ 2.36 (s, 3H, CH₃), 2.39 (s, 3H, CH₃), 2.65 (s, 3H, CH₃), 4.20 (s, 2H, CH₂), 6.09–7.45 (m, 12H, CH-Ar), 9.43 (bs, 1H, NH), 10.62 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 20.90, 21.30, 21.50, 40.10, 105.20, 112.40, 113.50, 113.80, 115.70, 116.10, 122.40, 123.20, 127.40, 129.20, 129.90, 134.50, 135.30, 136.10, 150.20, 154.40, 155.10, 162.10, 163.40, 165.20, 168.80, 170.10; MS m/z (%): 524 [M^+] (90). Anal. calcd for $C_{30}H_{25}FN_4O_2S$: C, 68.68; H, 4.80; N, 10.68. Found: C, 68.65; H, 4.82; N, 10.66.

5.1.4.3. 5-Cyano-4-(2,5-dimethoxyphenyl)-*N*-(4-fluorophenyl)-6-((2-(4-fluorophenyl) amino)-2-oxoethyl)thio)-2-methylnicotinamide (7c). Yellow solid (88%); mp: 230–233 °C (EtOH); IR (KBr, ν , cm^{-1}): 3351 (NH), 3277 (NH), 2206 (CN), 1668 (C=O), 1644 (C=O); 1H NMR (DMSO- d_6): δ 2.65 (s, 3H, CH₃), 3.68 (s, 3H, OCH₃), 3.69 (s, 3H, OCH₃), 4.21 (s, 2H, CH₂), 7.01–7.92 (m, 11H, CH-Ar), 9.45 (bs, 1H, NH), 10.39 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 20.90, 39.10, 56.10, 56.80, 104.20, 112.50, 113.40, 113.90, 115.60, 116.20, 122.30, 123.10, 127.20, 128.30, 129.50, 134.20, 135.50, 136.70, 150.20, 153.90, 155.20, 161.40, 163.10, 164.90, 167.80, 169.80; MS m/z (%): 574 [M^+] (47). Anal. calcd for $C_{30}H_{24}F_2N_4O_4S$: C, 62.71; H, 4.21; N, 9.75. Found: C, 62.69; H, 4.22; N, 9.73.

5.1.4.4. 5-Cyano-*N*-(4-fluorophenyl)-6-((2-(4-fluorophenyl)amino)-2-oxoethyl)thio)-2-methyl-4-(*p*-tolyl)nicotinamide (7d). Pale yellow solid (84%); mp: 243–245 °C (EtOH); IR (KBr, ν , cm^{-1}): 3446 (NH), 3336 (NH), 2225 (CN), 1661 (C=O), 1641 (C=O); 1H NMR (DMSO- d_6): δ 2.36 (s, 3H, CH₃), 2.54 (s, 3H, CH₃), 4.15 (s, 2H, CH₂), 7.11–7.97 (m, 12H, CH-Ar), 9.46 (bs, 1H, NH), 10.63 (bs, 1H, NH); MS m/z (%): 528 [M^+] (95). Anal. calcd for $C_{29}H_{22}F_2N_4O_2S$: C, 65.90; H, 4.20; N, 10.60. Found: C, 65.92; H, 4.19; N, 10.63.

5.1.5. Preparation of Compounds 8a–8d. **5.1.5.1. Method A.** After 3 h of refluxing compound 7a–7d (10 mmol) in sodium ethoxide solution (0.23 g Na in 100 mL EtOH), a yellow precipitate was detected. The reaction was then allowed to cool before the produced precipitate was filtered, dried, and crystallized in the appropriate solvent to yield compounds 8a–8d.

5.1.5.2. Method B. For 3 h, a combination of compound 3a or 3b (10 mmol) was refluxed in sodium ethoxide solution ethoxide (0.23 g Na in 100 mL of EtOH) with the appropriate derivative of 2-chloro-*N*-arylacetamide (10 mmol). After observing a yellow precipitate, the mixture was allowed to cool. The produced solid was filtered out, dried, and crystallized from ethanol.

5.1.5.3. 3-Amino-4-(2,5-dimethoxyphenyl)-*N*-(4-fluorophenyl)-6-methyl-*N*-(*p*-tolyl) thieno[2,3-*b*]pyridine-2,5-dicarboxamide (8a). Yellow solid [A] (91%), [B] (79%); mp: 236–238 °C (EtOH). IR (KBr, ν , cm^{-1}): 3477–3397 (NH₂), 3340 (NH), 3318 (NH), 1650 (C=O), 1633 (C=O). 1H NMR (CDCl₃): δ 1.87 (s, 3H, CH₃), 2.72 (s, 3H, CH₃), 3.71 (s, 3H, OCH₃), 3.82 (s, 3H, OCH₃), 5.84 (bs, 2H, NH₂), 6.95–7.87 (m, 11H, CH-Ar), 9.52 (bs, 1H, NH), 10.24 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 20.80, 21.20, 55.90, 56.20, 112.40,

113.50, 114.80, 116.10, 122.50, 123.40, 126.90, 127.80, 128.20, 129.30, 132.50, 134.50, 135.50, 136.70, 150.90, 154.10, 155.60, 157.10, 161.80, 163.90, 168.10, 170.80; MS m/z (%): 570 [M^+] (58). Anal. calcd for $C_{31}H_{27}FN_4O_4S$: C, 65.25; H, 4.77; N, 9.82. Found: C, 65.28; H, 4.79; N, 9.80.

5.1.5.4. 3-Amino-*N*-(4-fluorophenyl)-6-methyl-*N*,4-di-*p*-tolylthieno[2,3-*b*]pyridine-2,5-dicarboxamide (8b). Pale yellow solid [A] 92%, [B] 75%; mp: 245–247 °C (EtOH); IR (KBr, ν , cm^{-1}): 3532–3468 (NH₂), 3342 (NH), 3315 (NH), 1655 (C=O), 1631 (C=O); 1H NMR (DMSO- d_6): δ 2.27 (s, 6H, 2CH₃), 2.61 (s, 3H, CH₃), 5.76 (bs, 2H, NH₂), 7.13–7.61 (m, 12H, CH-Ar), 9.53 (bs, 1H, NH), 10.38 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 20.80, 21.20, 21.60, 112.20, 113.70, 115.40, 116.30, 122.30, 123.20, 127.60, 128.30, 129.10, 129.80, 133.60, 134.40, 135.60, 136.30, 151.10, 153.90, 155.30, 158.90, 163.50, 164.80, 167.90, 171.20; MS m/z (%): 524 [M^+] (16). Anal. calcd for $C_{30}H_{25}FN_4O_2S$: C, 68.68; H, 4.80; N, 10.68. Found: C, 68.70; H, 4.78; N, 10.71.

5.1.5.5. 3-Amino-4-(2,5-dimethoxyphenyl)-*N*,*N*-bis(4-fluorophenyl)-6-methylthieno[2,3-*b*]pyridine-2,5-dicarboxamide (8c). Yellow solid [A] 91%, [B] 72%; mp: 240–243 °C (EtOH); IR (KBr, ν , cm^{-1}): 3467–3369 (NH₂), 3328 (NH), 3146 (NH), 1673 (C=O), 1626 (C=O); 1H NMR (DMSO- d_6): δ 2.79 (s, 3H, CH₃), 3.71 (s, 3H, OCH₃), 3.97 (s, 3H, OCH₃), 5.73 (bs, 2H, NH₂), 6.79–7.83 (m, 11H, CH-Ar), 9.66 (bs, 1H, NH), 10.56 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ : 22.33, 56.08, 56.72, 97.69, 115.42, 115.80, 115.97, 116.00, 116.01, 116.03, 121.85, 121.92, 123.79, 123.85, 131.01, 135.30, 135.31, 135.33, 135.55, 135.57, 135.58, 140.90, 147.67, 150.76, 153.20, 155.97, 158.95, 159.88, 164.30, 165.30; MS m/z (%): 574 [M^+] (7), 464 (18), 137 (100), 109 (65), 82 (35). Anal. calcd for $C_{30}H_{24}F_2N_4O_4S$: C, 62.71; H, 4.21; N, 9.75. Found: C, 62.72; H, 4.23; N, 9.73.

5.1.5.6. 3-Amino-*N*,*N*-bis(4-fluorophenyl)-6-methyl-4-(*p*-tolyl)thieno[2,3-*b*]pyridine-2,5-dicarboxamide (8d). Yellow solid [A] 90%, [B] 74%; mp: 225–228 °C (EtOH); IR (KBr, ν , cm^{-1}): 3526–3469 (NH₂), 3343 (NH), 3305 (NH), 1654 (C=O), 1632 (C=O); 1H NMR (DMSO- d_6): δ 2.33 (s, 3H, CH₃), 2.63 (s, 3H, CH₃), 5.77 (bs, 2H, NH₂), 7.07–7.66 (m, 12H, CH-Ar), 9.57 (bs, 1H, NH), 10.39 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ : 21.41, 22.98, 97.77, 115.41, 115.58, 115.77, 115.95, 116.00, 116.01, 116.03, 122.0, 122.07, 123.79, 123.85, 129.0, 129.48, 130.98, 131.01, 135.30, 135.33, 135.55, 135.57, 135.58, 143.98, 147.44, 155.51, 159.19, 164.27, 165.44; MS m/z (%): 528 [M^+] (16). Anal. calcd for $C_{29}H_{22}F_2N_4O_2S$: C, 65.90; H, 4.20; N, 10.60. Found: C, 65.92; H, 4.23; N, 10.57.

5.1.6. Preparation of Compounds 10a–10e. A few drops of trimethylamine were added to a combination of 3a or 3b (10 mmol) and ethyl 2-(2-phenylhydrazono)-2-chloroacetate 9a or 9b (10 mmol) in absolute ethanol (15 mL). After 3 h of reflux, the reaction mixture was allowed to cool. Compounds 10a–10e were obtained by filtering the produced precipitate, drying, and crystallizing it in the appropriate solvent.

5.1.6.1. 3-Cyano-4-(2,5-dimethoxyphenyl)-5-((4-fluorophenyl)carbonyl)-6-methylpyridin-2-yl-2-oxo-*N*-phenylpropanehydrazonothioate (10a). Yellow solid (90%); mp 190–192 °C (EtOH); IR (KBr, ν , cm^{-1}): 3461 (NH), 3277 (NH), 2221 (CN), 1695 (C=O), 1650 (C=O); 1H NMR (DMSO- d_6): δ 2.51 (s, 3H, CH₃), 2.66 (s, 3H, CH₃), 3.66 (s, 3H, OCH₃), 3.68 (s, 3H, OCH₃), 7.04–7.79 (m, 12H, CH-Ar), 9.66 (bs, 1H, NH), 10.40 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 20.50, 54.80, 56.20, 104.50, 113.70, 114.40, 115.60, 115.90, 116.60, 121.90, 122.80, 127.50, 129.50, 130.10, 135.40, 136.70,

139.10, 150.20, 154.20, 155.90, 158.30, 162.60, 163.10, 165.10, 170.00, 193.50; MS m/z (%): 583 [M^+] (91). Anal. calcd for $C_{31}H_{26}FN_5O_4S$: C, 63.80; H, 4.49; N, 12.00. Found: C, 63.79; H, 4.51; N, 11.99.

5.1.6.2. 3-Cyano-4-(2,5-dimethoxyphenyl)-5-((4-fluorophenyl)carbamoyl)-6-methylpyridin-2-yl-2-oxo-N-(p-tolyl)propanehydrazonothioate (10b). Deep yellow solid (90%); mp 216–218 °C (EtOH); IR (KBr, ν , cm^{-1}): 3361 (NH), 3270 (NH), 2217 (CN), 1709 (C=O), 1653 (C=O); 1H NMR (DMSO- d_6): δ 2.48 (s, 3H, CH₃), 2.59 (s, 3H, CH₃), 2.67 (s, 3H, CH₃), 3.65 (s, 3H, OCH₃), 3.73 (s, 3H, OCH₃), 7.23–7.67 (m, 11H, CH-Ar), 9.71 (bs, 1H, NH), 10.92 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 20.70, 21.50, 55.90, 56.70, 104.20, 113.80, 114.60, 115.20, 115.70, 116.20, 122.80, 123.40, 128.10, 129.30, 129.90, 135.30, 136.90, 139.80, 150.50, 155.30, 156.50, 158.70, 162.40, 163.60, 165.50, 170.20, 194.80; MS m/z (%): 597 [M^+] (62). Anal. calcd for $C_{32}H_{28}FN_5O_4S$: C, 64.31; H, 4.72; N, 11.72. Found: C, 64.29; H, 4.74; N, 11.73.

5.1.6.3. 3-Cyano-5-((4-fluorophenyl)carbamoyl)-6-methyl-4-(p-tolyl)pyridin-2-yl-2-oxo-N-phenylpropanehydrazonothioate (10c). Yellow solid (90%); mp 205–207 °C (EtOH); IR (KBr, ν , cm^{-1}): 3461 (NH), 3277 (NH), 2218 (CN), 1718 (C=O), 1654 (C=O); 1H NMR (DMSO- d_6): δ 2.39 (s, 3H, CH₃), 2.53 (s, 3H, CH₃), 2.62 (s, 3H, CH₃), 6.95–7.64 (m, 13H, CH-Ar), 9.68 (bs, 1H, NH), 10.84 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 20.80, 21.70, 102.60, 113.20, 114.10, 115.80, 116.20, 118.20, 121.30, 123.10, 127.80, 129.10, 134.80, 136.10, 138.90, 148.20, 154.60, 155.20, 157.30, 158.40, 160.50, 162.50, 165.40, 169.90, 194.90; MS m/z (%): 537 [M^+] (54). Anal. calcd for $C_{30}H_{24}FN_5O_2S$: C, 67.02; H, 4.50; N, 13.03. Found: C, 67.04; H, 4.48; N, 13.05.

5.1.6.4. 3-Cyano-5-((4-fluorophenyl)carbamoyl)-6-methyl-4-(p-tolyl)pyridin-2-yl-2-oxo-N-(p-tolyl)propanehydrazonothioate (10d). Deep yellow solid (86%); mp 200–201 °C (EtOH); IR (KBr, ν , cm^{-1}): 3461 (NH), 3277 (NH), 2218 (CN), 1718 (C=O), 1654 (C=O); 1H NMR (DMSO- d_6): δ 2.34 (s, 3H, CH₃), 2.52 (s, 3H, CH₃), 2.55 (s, 3H, CH₃), 2.64 (s, 3H, CH₃), 7.13–7.89 (m, 12H, CH-Ar), 9.42 (bs, 1H, NH), 10.91 (bs, 1H, NH); MS m/z (%): 551 [M^+] (32). Anal. calcd for $C_{31}H_{26}FN_5O_2S$: C, 67.50; H, 4.75; N, 12.70; Found: C, 67.53; H, 4.73; N, 12.72.

5.1.6.5. 3-Cyano-4-(2,5-dimethoxyphenyl)-5-((4-fluorophenyl)carbamoyl)-6-methylpyridin-2-yl(E)-N-(4-fluorophenyl)-2-oxopropanehydrazonothioate (10e). Yellow solid (87%); mp 220–222 °C (EtOH); IR (KBr, ν , cm^{-1}): 3259 (NH), 3235 (NH), 2221 (CN), 1676 (C=O), 1650 (C=O); 1H NMR (DMSO- d_6): δ 2.43 (s, 3H, CH₃), 2.66 (s, 3H, CH₃), 3.63 (s, 3H, OCH₃), 3.71 (s, 3H, OCH₃), 7.12–7.76 (m, 11H, CH-Ar), 9.51 (bs, 1H, NH), 10.62 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 20.80, 56.10, 57.30, 103.40, 113.70, 114.10, 115.10, 115.80, 117.10, 123.20, 124.30, 127.50, 128.90, 129.30, 134.50, 136.30, 138.70, 150.00, 155.90, 157.10, 158.10, 160.40, 162.50, 164.30, 169.50, 195.50; MS m/z (%): 601 [M^+] (85), 538 (62), 405 (100). Anal. Calcd for $C_{31}H_{25}F_2N_5O_4S$: C, 61.89; H, 4.19; N, 11.64. Found: C, 61.85; H, 4.21; N, 11.67.

5.1.7. Preparation of Compounds 12a or 12b.

5.1.7.1. Method A. After adding methyl iodide, compound **3a** or **3b** (10 mmol) in sodium ethoxide solution (0.23 g Na in 100 mL of EtOH) was refluxed for 3–6 h (15 mmol). The methylthio nicotinamide derivatives **12a** and **12b** were obtained by filtering the solid generated after cooling, drying, and crystallizing it from the specified solvent.

5.1.7.2. Method B. Methyl iodide (15 mmol) was added to a stirred solution of compound **3a** or **3b** (10 mmol) and potassium hydroxide (10 mmol) in dichloromethane (75 mL) and stirred for another 6 h at room temperature. The resultant precipitate was filtered and recrystallized from ethanol to provide the methylthio nicotinamide derivatives **12a** and **12b**, respectively.

5.1.7.3. 5-Cyano-4-(2,5-dimethoxyphenyl)-N-(4-fluorophenyl)-2-methyl-6-(methylthio)-nicotinamide (12a). Yellow solid [A] 91%; [B] 88%; mp: 190–193 °C (EtOH); IR (KBr, ν , cm^{-1}): 3245 (NH), 2222 (CN), 1638 (C=O); 1H NMR (DMSO- d_6): δ 2.62 (s, 3H, CH₃), 2.69 (s, 3H, CH₃), 3.96 (s, 3H, OCH₃), 3.87 (s, 3H, OCH₃), 6.94–7.48 (m, 7H, CH-Ar), 10.49 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 13.38, 23.58, 55.91, 56.53, 105.59, 113.39, 115.88, 116.01, 116.72, 121.73, 121.78, 123.61, 129.62, 135.09, 148.95, 150.45, 153.05, 158.23, 158.67, 159.60, 162.03, 164.06; MS m/z (%): 437 [M^+] (15), 327 (22), 312 (60), 281 (100). Anal. calcd for $C_{23}H_{20}FN_3O_3S$: C, 63.15; H, 4.61; N, 9.61. Found: C, 63.17; H, 4.59; N, 9.63.

5.1.7.4. 5-Cyano-N-(4-fluorophenyl)-2-methyl-6-(methylthio)-4-(p-tolyl)-nicotinamide (12b). Pale yellow solid [A] 93%; [B] 89%; mp: 95–99 °C (EtOH); IR (KBr, ν , cm^{-1}): 3245 (NH), 2222 (CN), 1638 (C=O); 1H NMR (DMSO- d_6): δ 2.32 (s, 3H, CH₃), 2.49 (s, 3H, CH₃), 2.63 (s, 3H, CH₃), 7.06–7.40 (m, 8H, CH-Ar), 10.42 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 13.38, 21.10, 22.10, 105.40, 113.40, 115.90, 116.20, 116.60, 121.60, 122.10, 123.20, 128.50, 134.10, 146.80, 149.50, 152.90, 157.30, 159.80, 160.10, 163.20, 164.50; MS m/z (%): 391 [M^+] (5), 352 (10), 281 (100), 83 (57). Anal. calcd for $C_{22}H_{18}FN_3OS$: C, 67.50; H, 4.63; N, 10.73. Found: C, 67.51; H, 4.60; N, 10.71.

5.1.8. Preparation of Compounds 13 and 14. Compound **3a** or **3b** (10 mmol) was refluxed with 2-bromopropionate or 3-chloro-2,4-pentandione (10 mmol) in the presence of absolute ethanol (75 mL) and few drops of TEA as a catalyst for 3–6 h. The solid formed after cooling was filtered off, dried, and crystallized from the specified solvent to afford the corresponding nicotinamide derivatives **13** and **14** respectively.

5.1.8.1. Ethyl-2-((3-cyano-4-(2,5-dimethoxyphenyl)-5-((4-fluorophenyl)carbamoyl)-6-methylpyridin-2-yl)thio)propanoate (13a). Pale yellow solid (81%); mp 240–244 °C (EtOH); IR (KBr, ν , cm^{-1}): 3243 (NH), 2221 (CN), 1742 (C=O), 1652 (C=O); 1H NMR (DMSO- d_6): δ 1.26 (t, 3H, CH₃), 2.16 (s, 3H, CH₃), 2.55 (s, 3H, CH₃), 3.70 (s, 3H, OCH₃), 3.74 (s, 3H, OCH₃), 4.19 (q, 2H, CH₂), 4.62 (q, 1H, CH), 6.95–7.46 (m, 7H, CH-Ar), 10.52 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 13.90, 21.10, 56.10, 56.90, 61.30, 103.83, 115.30, 115.90, 116.70, 120.90, 121.80, 127.30, 128.50, 129.80, 129.50, 130.10, 131.50, 135.10, 138.90, 150.70, 157.40, 158.90, 159.60, 161.20, 164.50, 170.90; MS m/z (%): 523 [M^+] (63). Anal. calcd for $C_{27}H_{26}FN_3O_5S$: C, 61.94; H, 5.01; N, 8.03. Found: C, 61.92; H, 4.99; N, 8.06.

5.1.8.2. Ethyl-2-((3-cyano-5-((4-fluorophenyl)carbamoyl)-6-methyl-4-(p-tolyl)pyridin-2-yl)thio)propanoate (13b). Yellow solid (88%); mp 210–212 °C (EtOH); IR (KBr, ν , cm^{-1}): 3238 (NH), 2217 (CN), 1725 (C=O), 1648 (C=O); 1H NMR (DMSO- d_6): δ 1.23 (t, 3H, CH₃), 2.18 (s, 3H, CH₃), 2.44 (s, 3H, CH₃), 2.59 (s, 3H, CH₃), 4.20 (q, 2H, CH₂), 4.65 (q, 1H, CH), 7.09–7.48 (m, 8H, CH-Ar), 10.47 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ 14.50, 18.99, 21.28, 23.10, 61.72, 103.92, 115.20, 115.86, 115.97, 121.92, 121.97, 128.24, 128.74, 129.37, 129.49, 129.52, 131.46, 134.90, 139.93, 151.86, 158.33, 158.58, 159.70, 160.96, 164.13, 171.87; MS m/z (%): 477 [M^+] (78),

433 (58), 323 (100), 281 (48); MS m/z (%): 477 [M^+] (78), 433 (58), 323 (100), 281 (48). Anal. calcd for $C_{26}H_{24}FN_3O_3S$: C, 65.39; H, 5.07; N, 8.80. Found: C, 65.43; H, 5.09; N, 8.79.

5.1.8.3. 5-Cyano-4-(2,5-dimethoxyphenyl)-6-((2,4-dioxopentan-3-yl)thio)-N-(4-fluorophenyl)-2-methylnicotinamide (14a). Pale yellow solid (81%); mp 188–192 °C (EtOH); IR (KBr, ν , cm^{-1}): 3342 (NH), 2219 (CN), 1728 (C=O), 1688 (C=O), 1650 (C=O); 1H NMR (DMSO- d_6): δ 2.34 (s, 6H, 2CH₃), 2.61 (s, 3H, CH₃), 3.65 (s, 3H, OCH₃), 3.69 (s, 3H, OCH₃), 4.65 (s, 1H, CH), 7.11–7.82 (m, 7H, CH-Ar), 10.50 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ : 21.30, 26.90, 55.90, 56.20, 78.10, 103.83, 112.90, 113.30, 113.60, 115.90, 116.70, 123.10, 127.30, 133.60, 150.40, 153.20, 153.80, 154.90, 159.60, 162.30, 163.20, 164.80, 170.10, 205.90; MS m/z (%): 521 [M^+] (42). Anal. calcd for $C_{27}H_{24}FN_3O_5S$: C, 62.18; H, 4.64; N, 8.06. Found: C, 62.15; H, 4.66; N, 8.08.

5.1.8.4. 5-Cyano-6-((2,4-dioxopentan-3-yl)thio)-N-(4-fluorophenyl)-2-methyl-4-(p-tolyl)nicotinamide (14b). Yellow solid (85%); mp 212–216 °C (EtOH); IR (KBr, ν , cm^{-1}): 3258 (NH), 2223 (CN), 1712 (C=O), 1696 (C=O), 1641 (C=O); 1H NMR (DMSO- d_6): δ 2.34 (s, 6H, 2CH₃), 2.51 (s, 3H, CH₃), 2.63 (s, 3H, CH₃), 4.65 (s, 1H, CH), 6.14–7.49 (m, 8H, CH-Ar), 10.47 (bs, 1H, NH); ^{13}C NMR (DMSO- d_6): δ : 20.90, 27.10, 77.90, 103.60, 112.20, 113.10, 113.80, 115.50, 116.80, 123.50, 127.40, 133.20, 150.60, 153.10, 153.60, 154.80, 160.10, 162.50, 163.90, 165.70, 169.10, 206.10; MS m/z (%): 476 [$M^+ + 1$] (39), 462 (45), 367 (100), 293 (100). Anal. calcd for $C_{26}H_{22}FN_3O_3S$: C, 65.67; H, 4.66; N, 8.84. Found: C, 65.64; H, 4.69; N, 8.80.

5.2. Antiproliferative Activity. **5.2.1. Cell Culture Conditions.** Human colorectal carcinoma (HCT-116), human liver carcinoma (HepG-2), human breast adenocarcinoma (MCF-7), and normal human skin fibroblast (BJ-1) cell lines were purchased from the American Type Culture Collection (Rockville, MD) and maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U mL⁻¹ penicillin, and 100 U mL⁻¹ streptomycin. The cells were grown at 37 °C in a humidified atmosphere of 5% CO₂.

5.2.2. MTT Antiproliferative Assay. The antiproliferative properties of the compounds were assessed using the 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) test against HepG-2, HCT-116, MCF-7, and BJ-1 cells. MTT cleavage by mitochondrial dehydrogenases in live cells is used in this test.^{37–39} Cells were seeded in a 96-well sterile microplate (5×10^4 cells well⁻¹) and incubated for 48 h at 37 °C in serum-free media containing dimethyl sulfoxide (DMSO) and either a series of different concentrations of each test compound or doxorubicin (as a positive control) before the MTT assay. After incubation, the medium was withdrawn and each well was filled with 40 μ L of MTT (2.5 mg mL⁻¹). The incubation period was extended for another 4 h. The purple formazan dye crystals that resulted were solubilized in 200 μ L of DMSO. In a Spectra Max Paradigm Multi-Mode microplate reader, absorbance was measured at 590 nm (Molecular Devices, LLC, San Jose, CA). The mean proportion of viable cells compared to untreated control cells was used to calculate relative cell viability. Technical triplicates and three biological duplicates were used in all studies. All data was presented as a mean standard deviation. SPSS Inc. determined the IC₅₀ values. Probit analysis was used to determine the IC₅₀ values (IBM Corp., Armonk, NY).

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Notes

The authors declare no competing financial interest.

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