Received: 6 April 2021 | Revised: 5 May 2021 | Accepted: 27 May 2021

DOI: 10.1002/jhet.4310

ARTICLE

JOURNAL OF HETEROCYCLIC CHEMISTRY WILEY

Synthesis, characterization, and biological activities of some novel thienylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidines and related heterocycles

Suzan Abuelhassan | Etify A.-G. Bakhite | Abdu E. Abdel-Rahman | Ahmed F. M. El-Mahdy

Department of Chemistry, Faculty of Science, Assiut University, Assiut, Egypt

Correspondence
Etify A.-G. Bakhite, Department of Chemistry, Faculty of Science, Assiut University, Assiut 71516, Egypt.
Email: etafy@aun.edu.eg

Abstract

3-Cyano-5-ethoxycarbonyl-6-methyl-4-(2′-thienyl)-pyridine-2(1*H*)-thione (**1**) is synthesized and reacted with chloroacetamide or chloroacetonitrile to give 3-amino-5-ethoxycarbonyl-6-methyl-4(2′-thienyl)-thieno[2,3-*b*]pyridine-2-carboxamide **3a** or its 2-carbonitrile analog **3b**, respectively. Cyclocondensation of **3a** with triethylorthoformate produced the corresponding pyridothienopyrimidineone **4**, which on heating with phosphorus oxychloride gave 4-chloropyrimidine derivative **5**. Compound **5** was used as key intermediate for synthesizing compounds **6**, **9**, **10**, **11**, and **12** upon treatment with some nucleophilic reagents such as thiourea, 5-phenyl-*s*-triazole-3(1*H*)-thione, piperidine, morpholine, or hydrazine hydrate, respectively. Reaction of pyridothienopyrimidinethione **6** with *N*-(4-tolyl)-2-chloroacetamide or ethyl bromoacetate afforded the corresponding *S*-substituted methylsulfanylpyrimidines **7** or **8**. The condensation of **3b** with triethylorthoformate gave azomethine derivative **13**, which was reacted with hydrazine hydrate to give ethyl 3-amino-3,4-dihydro-4-imino-7-methyl-9-(2′-thienyl) pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine-8-carboxylate (**14**). Compounds **12** and **14** were used as precursors for synthesizing other new thienylpyridothienopyrimidines as well as isomeric thienyl-*s*-triazolopyridothieno- pyrimidines. All synthesized compounds were characterized by elemental and spectral analyses such as IR, ^{1}H NMR, and ^{13}C NMR. In addition, majority of synthesized compounds were tested for their antifungal activity against five strains of fungi. Moreover, compounds **3a**, **5**, **6**, **8**, and **22** were screened for their anticancer activity against HEPG-2 and MCF-7 cell lines.

1 | INTRODUCTION

Sulfur-containing heterocycles opened the way for the active research in the pharmaceutical chemistry. Nowadays thiophene and its derivatives are an essential class of heterocyclic compounds that have a wide range of biological applications such as anti-inflammatory [1], anticonvulsant [2], antibacterial [3], antitumor [4], antifungal [5], anti-parasitic [6], antiviral [7], anti-nociceptive [8], DNA cleavage [9], herbicidal [10], anti-tubercular [11], protein kinase inhibition [12], and respiratory syndrome protease inactivation [13].

Numerous pyridine-containing natural products and synthetic organic compounds have diverse biophysio- and pharmacological activity [14–18]. These scaffolds are also of wide spread interest in supramolecular and coordination chemistry, as well as for materials science [19]. Thieno[2,3-*b*]pyridines have received considerable

attention since they show a wide variety of bioactivity, for example, antiviral [20,21], antidiabetic [22], antimicrobial [23–25], antitumor [26], antiparasitic [27], and neurotropic [28]. Other thienopyridine modifies have important pharmacologically activities including antiplatelet drugs for the treatment of acute coronary syndromes [29,30], antibacterial activity against a drug-resistant *S. epidermidis* clinical strain [31], and cytotoxic activity against human hepatocellular liver carcinoma (HepG2) [32]. Actually, the key unit of thieno[2,3-*b*]pyridines is found as the central fragment in many clinical pharmaceuticals [33,34]. The pharmacological importance of thieno[2,3-*b*]pyridines has directed considerable research activity toward the construction of the skeleton of such heterocycles [35–37].

In view of the aforementioned findings, the present project was designed to synthesize and characterize of some new thiophene-tagged thieno[2,3-*b*]pyridines, pyridothienopyrimidine, and *s*-triazolopyridothienopyrimidine hoping to get novel condensed polycyclic compounds with anticipated biological and medicinal importance. In addition, majority of synthesized compounds were tested for their antifungal activity against five strains of fungi. Moreover, compounds **3a**, **5**, **6**, **8**, and **22** were screened for their anticancer activity against HEPG-2 and MCF-7 cell lines.

2 | RESULTS AND DISCUSSION

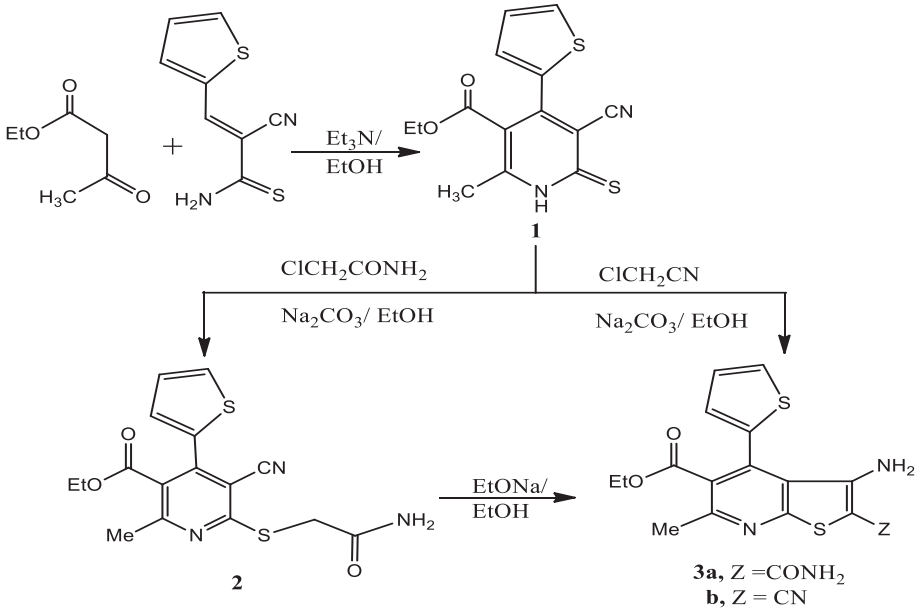
2.1 | Synthesis and characterization

Our approach to the synthesis of the target compounds started from 3-cyano-5-ethoxycarbonyl-6-methyl-4-(2′-thienyl)pyridine-2-(1*H*)-thione (**1**), which was prepared from the reaction of 2-cyano-3-(2′-thienyl)prop-2-enethioamide with ethyl acetoacetate in the presence of triethylamine according to the reported procedure (Scheme 1) [38]. All characterization data of compound **1** are in agreement with those reported before [38].

Reaction of cyanopyridinethione **1** with chloroacetamide by refluxing in ethanol containing anhydrous sodium carbonate for 30 min gave [3-cyano-5-ethoxycarbonyl-6-methyl-4-(2′-thienyl)pyridin-2-ylthio]acetamide (**2**). In contrast, reaction of **1** with chloroacetonitrile under the same conditions furnished 3-amino-5-ethoxycarbonyl-6-methyl-4-(2′-thienyl)thieno[2,3-*b*]pyridine-2-carbonitrile (**3b**). Upon treatment of compound **2** with a catalytic amount of sodium ethoxide in ethanol, it underwent intramolecular Thorpe-Ziegler cyclization affording 3-amino-5-ethoxycarbonyl-6-methyl-4-(2′-thienyl)thieno[2,3-*b*] pyridine-2-carboxamide (**3a**) (Scheme 1).

IR spectrum of compound **2** showed characteristic absorption bands in the regions 3413–3176 cm^{-1} for (NH_2), 2223 cm^{-1} for (C≡N), and 1681 cm^{-1} for (C═O, amide). While that of its isomer **3a** revealed the disappearance of ν C≡N band. ^1H NMR spectrum of compound **2** showed the presence of a singlet signal corresponds to SCH_2 group at δ 4.02, which disappeared in the spectrum of **3a**, which exhibits instead of a singlet signal at δ 5.88 corresponds to NH_2 group attached to thiophene ring directly. IR spectrum of compound **3b** revealed the presence of three absorption bands in the region 3480–3125 cm^{-1} characteristic for NH_2 group and a band at 2198 cm^{-1} for carbonitrile group.

Cyclocondensation reaction of compound **3a** with triethyl orthoformate afforded pyridothienopyrimidineone

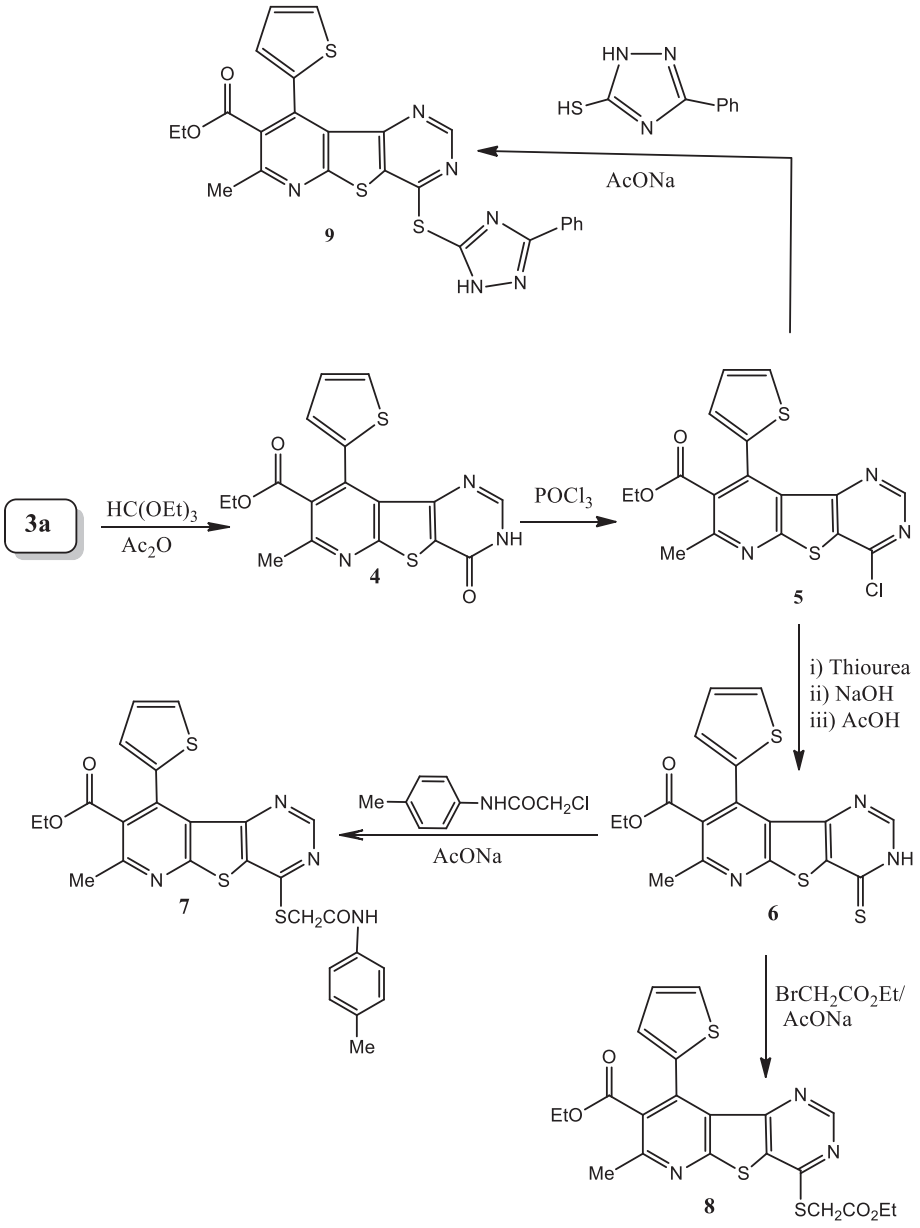


SCHEME 1 Synthesis of compounds **1**, **2**, and **3a**,**b**

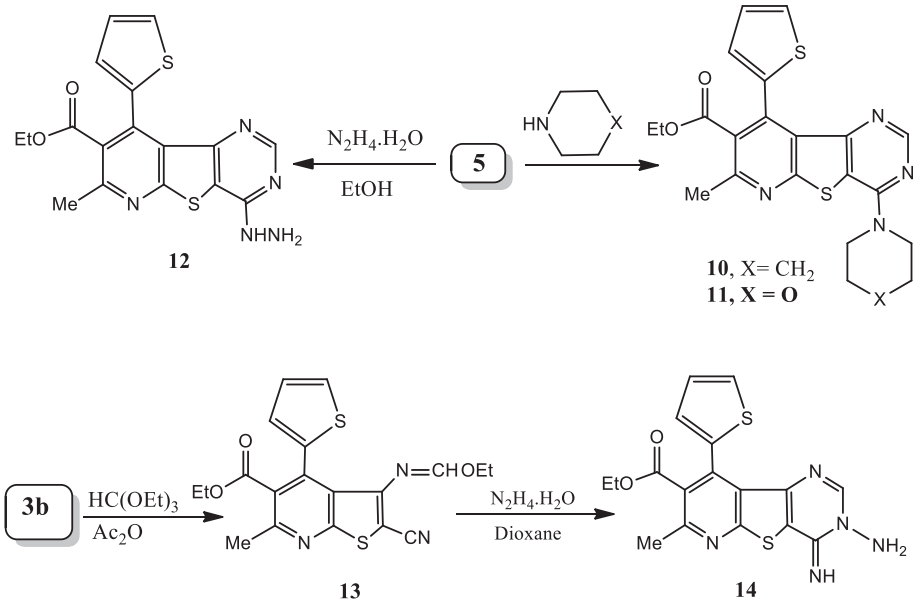
derivative **4**. Heating compound **4** with an excess amount of phosphorous oxychloride under neat conditions afforded 4-chloropyrimidine **5**, which was considered a good synthon for other thienylpyridothienopyrimidines. Thus, the interaction of compound **5** with thiourea gave an adduct, which on treatment with sodium hydroxide solution followed by acidification with acetic acid furnished pyrimidinethione **6** (Scheme 2).

IR spectrum of **4** showed the absence of the bands of the two amino groups and presence of two bands at 3165 cm^{-1} and 1662 cm^{-1} characteristic for NH and C═O groups of pyrimidineone **4**, respectively. ^{1}H NMR spectrum of **4** displayed two singlet signals at δ 11.25 and δ 8.19 correspond to NH and pyrimidine-H, respectively. IR spectrum of **5** revealed the absence of all aforementioned bands of compound **4**, and its ^{1}H NMR spectrum revealed the disappearance of the singlet signal at δ 11.25 (NH) of compound **4**. IR spectrum of compound **6** showed the presence of a band at 3279 cm^{-1} characteristic for NH group. ^{1}H NMR spectrum of **6** displayed a singlet signal at δ 14.40 corresponds to NH group.

On refluxing of the latter pyrimidinethione with some α-halocarbonyl compounds such as *N*-(4-tolyl)-2-chloroacetamide or ethyl bromoacetate in ethanol containing sodium acetate for 2 h, the corresponding *S*-substituted methylsulfanylpyridothienopyrimidines **7** and **8** were obtained in good yields (Scheme 2). In contrast, compound **5** reacted with 5-phenyl-3-mercapto-2*H*-1,2,4-triazole to give 3-[8-ethoxycarbonyl-7-methyl-9-(2′-thienyl) pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-ylsulfanyl]-5-phenyl-2*H*-1,2,4-triazole (**9**) (Scheme 2).



SCHEME 2 Synthesis of compounds **4–9**



SCHEME 3 Synthesis of compounds **10–14**

IR spectrum of **7** exhibited two bands at 3248 cm^{-1} characteristic for NH group and at 1687 cm^{-1} for C═O of anilide group. ^{1}H NMR spectrum of **7** showed two singlet signals at δ 9.37 and δ 4.09 corresponding to NH and SCH_2, respectively. IR spectrum of **8** showed the presence of a band at 1724 cm^{-1} characteristic for a non conjugated ester. ^{1}H NMR spectrum of **8** showed the presence of a singlet signal at δ 4.14 corresponding to SCH_2 besides other two signals equivalent to ethoxy group of the non conjugated ester. IR spectrum of **9** revealed the presence of a band at 3203 cm^{-1} characteristic for NH function of triazole ring. ^{1}H NMR spectrum of **9** exhibited a broad singlet signal at δ 15.20 referred to NH group.

Compound **5** underwent other nucleophilic displacement reactions when treated with piperidine or morpholine to furnish 4-substituted-8-ethoxycarbonyl-7-methyl-9-(2′-thienyl)pyrido[3′,2′:4,5]thieno[2,3-*d*]pyrimidines **10** and **11**, respectively (Scheme 3). Compound **12** also produced an excellent yield via condensation of chloropyrimidine **5** with hydrazine hydrate wherein only a nucleophilic substitution of the labile chlorine atom by hydrazino group occurred (Scheme 3).

^{1}H NMR spectrum of **11** showed a triplet signal at δ 3.93–3.95 equivalent to CH_2OCH_2 residue and a triplet signal at δ 3.85–3.87 equivalent to CH_2NCH_2 residue of morpholine ring. IR spectrum of **12** exhibited characteristic absorption bands in the region 3341–3188 cm^{-1} for ($NHNH_2$). ^{1}H NMR spectrum of **12** displayed three singlet signals at δ 9.14, δ 8.17, and δ 4.95 correspond to NH, pyrimidine-H, and NH_2 protons, respectively.

In contrast, condensation of *o*-aminocarbonitrile **3b** with triethyl orthoformate by refluxing in acetic anhydride led to the formation of azomethine **13**, which on stirring with hydrazine hydrate in dioxane converted into ethyl 3-amino-3,4-dihydro-4-imino-7-methyl-9-(2′-thienyl) pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine-8-carboxylate (**14**) (Scheme 3). The ester function of compounds **5** and **12–14** did not get affected by hydrazine hydrate under the applied conditions.

TABLE 1 Heat of formation of compounds **15–18**

Compd. No.	E (k.cal./Mol)	Compd. No.	E (k.cal./Mol)
15	510.7438	**16**	493.6434
17	509.2006	**18**	486.8352

IR spectrum of **13** proved the disappearance of the amino group of **3b** and presence of a band at 2212 cm^{-1} characteristic for (C≡N). IR spectrum of **14** showed characteristic absorption bands in the region 3313–3154 cm^{-1} for (NH) and (NH_2) groups. ^{1}H NMR spectrum of **14** displayed two singlets at δ 10.20 and δ 5.74 due to NH and NH_2 groups, respectively.

Hydrazino compound **12** and its isomeric amino-imino one **14** were used as precursors for synthesizing other new thienylpyridothienopyrimidines as well as thienyl-*s*-triazolopyridothienopyrimidines.

Thus, heating compound **12** with formic acid under reflux for 4 h led to the formation of unexpected product identified as *s*-triazolo compound **16** rather than the expected isomer **15**. On the same approach, the reaction of **12** with glacial acetic acid furnished methyl-*s*-triazolo compound **18** rather than the alternative one **17**. From thermodynamic point of view [39] the heat of formation of compounds **16** and **18** are less than those of the corresponding isomers **15** and **17** as shown in Table 1. So, both compounds **16** and **18** seems to be more stable than

the corresponding isomers **15** and **17**. The probable mechanism of this reaction starts with the usual formation of compounds **15** and **17**, which underwent Dimroth rearrangement to give the more stable ones **16** and **18**, respectively (Scheme 4). The mechanism of Dimroth rearrangement is reported in our previous publication [40]. The structure of compounds **16** and **18** was also confirmed by independent syntheses via heating compound **14** with triethylorthoformate and/ or acetic anhydride, respectively (Scheme 4).

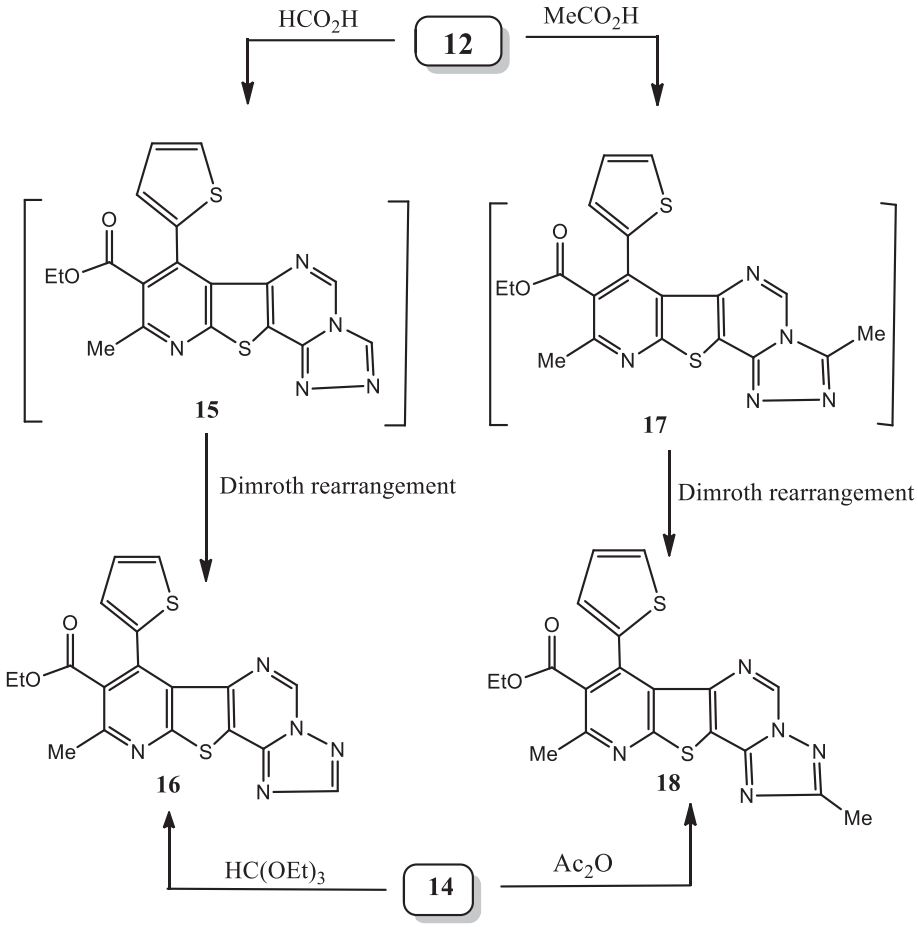
An attempt to synthesize *s*-triazolo derivative **15** in its pure state via condensation of hydrazino compound **12** with triethyl orthoformate in the presence of acetic anhydride has succeeded. In contrast, heating hydrazino compound **12** with acetic anhydride did not give the expected methyl-*s*-triazolo derivative **17** and instead of the triacetylated product **19** was isolated. The synthesis of **17** may need triethyl orthoacetate, which was not pursued at this time. However, heating compound **15** in formic acid furnished its isomer **16** via Dimroth rearrangement (Scheme 5).

IR spectrum of **15** revealed the disappearance of the hydrazino group of compound **12**. IR spectra of **16** and **18** revealed the disappearance of the hydrazino group of compound **12**, or amino and imino group of compound **14**. Both ^{1}H NMR spectrum of **15** and **16** showed a singlet signal at δ 8.85 or δ 8.46 corresponds to triazole-H, respectively. ^{1}H NMR spectrum of **18** displated a singlet signal at δ 2.65 corresponds to CH_3 attached to triazole moiety. IR spectrum of **19** showed a band at 1741 cm^{-1} due to (C═O, acetyl). ^{1}H NMR spectrum of **19** revealed the presence of two singlets at δ 2.53 and δ 2.47 correspond to two ($COCH_3$) and one ($COCH_3$), respectively.

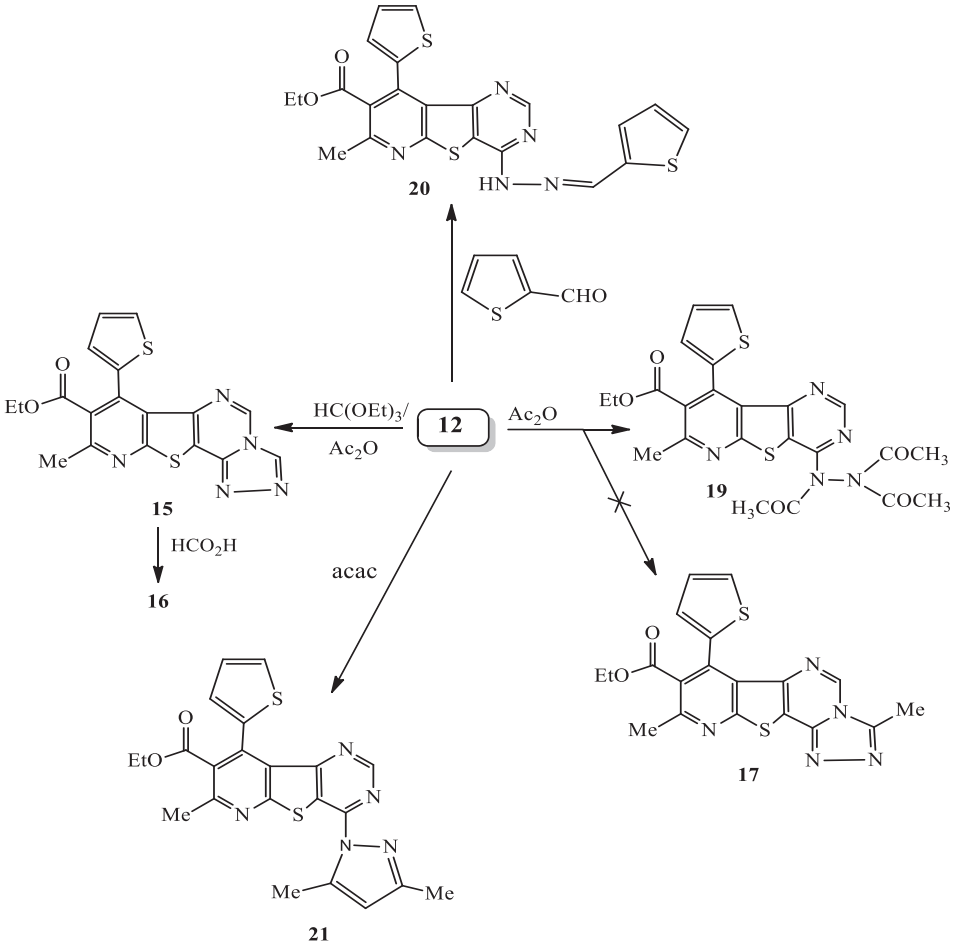
The condensation of hydrazino compound **12** with thiophene-2-carboxaldehyde gave the 4-(2′-thienyl)methylenehydrazinopyrimidine derivative **20**. In addition, cyclocondensation of **12** with acetylacetone under neat conditions gave 4-(3,5-dimethylpyrazol-1-yl)-8-ethox-ycarbonyl-7-methyl-9-(2′-thienyl)pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine (**21**) (Scheme 5).

^{1}H NMR spectrum of **20** showed the presence of two singlets at δ 10.92 and δ 8.43 equivalent to one proton (NH) and two protons (pyrimidine-H and N═CH), respectively. ^{1}H NMR of **21** displayed a singlet at δ 8.78 referred to pyrimidine-H and a singlet at δ 6.06 due to pyrazole-H.

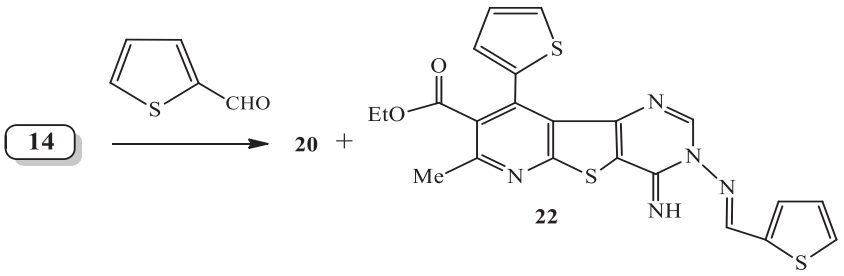
Condensation of compound **14** with thiophene-2-carboxaldehyde by refluxing in ethanol did not give



SCHEME 4 Synthesis of compounds **16** and **18**



SCHEME 5 Synthesis of compounds **15**, **16**, and **19–21**



SCHEME 6 Reaction of compound **14** with Thiophene-2-carboxaldehyde; formation of compounds **22** and **20**

mainly the expected compound **22** [40] nor its isomer **22** [41] but the product was assigned as a mixture of **22** and **20** in a 2:1 ratio. The formation of **20** is attributed to Dimroth rearrangement of **22** [41] (Scheme 6).

IR spectrum of **22** showed a band at 3245 cm^{-1} for (NH). ^1H NMR spectrum of **22** showed a singlet at δ 10.09 correspond to (NH), a singlet at δ 9.11 for pyrimidine-H, and a singlet at δ 8.50 for CH═N.

In general, the elemental analyses of all newly synthesized compounds gave satisfactory results within ±0.4 of the calculated values. IR spectra of these compounds showed characteristic absorption band in the region from 1731 to 1704 cm^{-1} for (C═O) of conjugated ester group attached to pyridine ring. This ester group appears in the ^1H NMR spectra of all synthesized compounds as a quartet signal at δ value ranged from 4.04–4.18 to 4.08–4.21 and a triplet signal at δ value ranged from 0.97–1.13 to 1.03–1.15. ^1H NMR spectra of all synthesized compounds exhibited a singlet signal at δ value ranged from 2.45 to 2.81 due to methyl group attached to pyridine ring. ^1H NMR spectra of compounds **5–11** showed the presence of a singlet signal at δ value ranged from 8.28 to 8.93 corresponds to pyrimidine-H. ^1H NMR spectra of compounds **14–16**, **18**, and **19** showed the presence of a singlet signal at δ value ranged from 7.89 to 9.16 corresponds to pyrimidine-H. ^{13}C NMR spectra of all synthesized compounds exhibited collections of peaks, which are in agreement with their proposed structures.

2.2 | Biological activity

2.2.1 | Antifungal activity

Resistance of various strains of the pathogenic bacteria and fungi to the current antimicrobial therapy has become one of the most threating problems worldwide. Therefore, the main target of our study is synthesis of new heterocyclic compounds having superior significance in biological and medicinal chemistry. Accordingly, majority of synthesized compounds were screened in vitro for their antifungal activity against five strains of fungi: *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Pencillium purpurogenum*, and *Candida albicans*.

The antifungal activities of the majority of synthesized compounds were examined as inhibition areas and were summarized in Table 2 and illustrated in Figure 1. From the data presented, it is clear that when compound **3a** reacted with triethyl orthoformate, the pyridothienopyrimidinone **4** that produced very high activity against *Aspergillus niger*, *Pencillium purpurogenum*, and *Candida albicans* with inhibition zones of 22 mm, 25 mm, and 25 mm, respectively, compared with the reference fungus Voriconazole, which posses inhibition zones of 20 mm, 17 mm, and 18 mm, respectively. Compound **4** showed high activity against *Aspergillus terreus* with inhibition zone of 18 mm, which is near to that of Voriconazole reference (20 mm). The product **13** that produced from the reaction of compound **3b** with triethyl orthoformate showed moderate effect against *Aspergillus flavus* and *Pencillium purpurogenum* with inhibition zones of 10 mm and 9 mm, respectively. Compounds **5** and **14** also have moderate effect against *Aspergillus flavus* and *Aspergillus terreus*. Compounds **7**, **10**, **19**, **20**, and **21** exhibited very high activity toward *Aspergillus niger* with inhibition zones of 24 mm, 21 mm, 20 mm, 20 mm, and 24 mm, respectively. Hydrazino compound **12** show very high activity against *Pencillium purpurogenum* with inhibition zone of 27 mm compared with Voriconazole reference (17 mm) and high activity against *Candida albicans* with zone diameter **16** mm, which near to the inhibition zone of the reference (18 mm). In addition, compounds **7** and **18** exhibited very high activity against *Aspergillus terreus* with inhibition zones of 20 mm and 25 mm compared with reference (20 mm) as well as compounds **19**, **20**, and **21**, which have great activity against the same fungus with inhibition zones of 18 mm, 19 mm, and 19 mm, respectively. Compounds **20** and **21** also exhibited very high activity against *Pencillium purpurogenum* and *Candida albicans* compared

TABLE 2 Comparison of inhibition zone diameters for the antifungal activity of compounds **3a**, **4–15**, and **18–22** with the Voriconazole reference (mm)

Code of sample	*A. flavus*	*A. niger*	*A. terreus*	*P. Purpurogenum*	*C. albicans*
3b	–	–	–	–	–
4	–	+22	+18	+25	+25
5	+15	–	+9	+22	+23
6	–	–	–	–	–
7	–	+24	+20	–	+25
8	–	–	–	–	–
9	–	–	–	–	–
10	–	+21	–	–	+15
11	–	–	–	–	–
12	–	–	–	+27	+16
13	+10	–	–	+9	–
14	+12	–	+11	–	–
15	–	–	–	–	–
18	–	–	+25	–	+25
19	–	+20	+18	+15	+12
20	–	+20	+19	+23	+23
21	–	+24	+19	+25	+24
122	–	–	–	–	–
Ref.	24	20	20	17	18

Note: (+) susceptibility. (–) absence of susceptibility (Resistance). Reference of fungus Voriconazole 10 mg/ml.

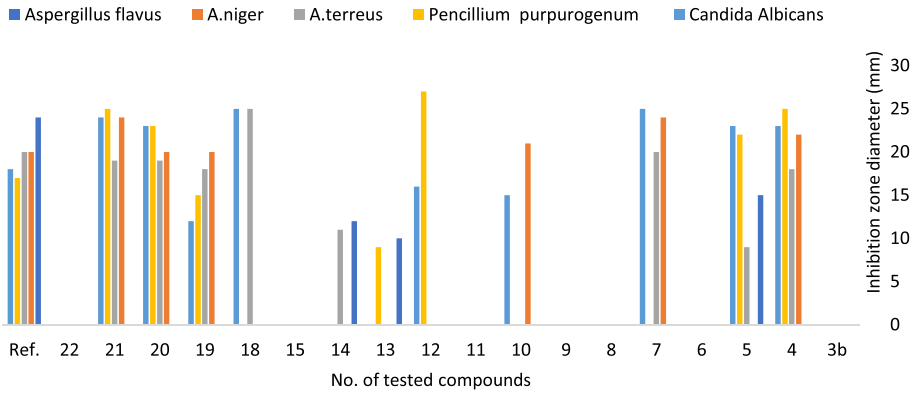


FIGURE 1 Comparison of inhibition zone diameters for the antifungal activity of the tested compounds

with compound **20**, which showed moderate activity against *Pencillium purpurogenum* and *Candida albicans*. Compound **7** showed very high activity against *Candida albicans* with inhibition zone of 25 mm compared with compound **10** which showed moderate activity with inhibition zone of 15 mm.

2.2.2 | Anticancer activity

Using the MTT assay compounds **3a**, **5**, **6**, **8**, and **22** were studied for their cytotoxic activity involving MCF-7 and HepG2 cell lines. As presented in Table 3 and Figures 2 and 3. It is clear from Table 3 that the thiophene moiety was found to be crucial for the cytotoxic effect of the choice cyclic products **3a**, **5**, **6**, **8**, and **22**. The selected compounds exhibited optimal cytotoxic effect against MCF-7 and HepG2 cell lines. With IC_{50} in the μg/ml range. The cytotoxicity of the product of *Thorpe-Ziegler* cyclization of compound **2** showed low cytotoxicity against MCF-7 and HepG2 cell lines. By comparing the cytotoxicity of compounds **5** and **6**, it is obvious that the cytotoxicity of **5** was higher than that of **6** against the two cell lines this may be due to the presence of chlorine atom that is responsible for its high potency. But when compound **6** reacted with ethyl bromoacetate, product **8** showed high potency against MCF-7 cell line compared with compound **6** this is due to the presence of enrich electron group COOEt that is responsible for its high potency. While the cytotoxicity of compound **22** showed moderate effect against the two cell lines. Results are given in concentrations that were able to cause 50% of cell growth inhibition (IC_{50}).

3 | EXPERIMENTAL

3.1 | Chemistry

Analytical-grade reagents and solvents for synthesis were obtained from Sigma Aldrich (USA). The chemicals were used as received (i.e., without further purification). The reactions were followed with TLC technique. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian A5 500 MHz spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C using $CDCl_3$ or DMSO-d_6 as a solvent and tetramethylsilane as internal standard. Chemical shifts are expressed in δ (ppm); coupling constants (*J* values) are given in Hertz (*Hz*). ^{1}H NMRs splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q), or multiples (m). FT-IR spectra were recorded on a Shimadzu 470 IR-spectrophotometer (KBr; ν_{max} in cm^{-1}). Microanalysis was performed using a Perkin Elmer 2400 LS Series CHN/O analyzer. Melting points were obtained using a Gallan-Kamp apparatus and were reported uncorrected. R_f values of all compounds were measured by using a mixture of benzene: ethyl acetate with different ratios as eluents.

3.1.1 | Synthesis of 3-cyano-5-ethoxcarbonyl-6-methyl-4-(2′-thienyl)pyridin-2-(1*H*)-thione (**1**)

To a mixture of 2-cyano-3-(2′-thienyl)prop-2-enethioamide (5.82 g, 30 mmol) and ethyl acetoacetate (3.90 ml, 30 mmol) in absolute ethanol (20 ml), triethylamine (1 ml) was added. The resulting mixture was heated under reflux for 5 h and then allowed to stand overnight. The orange crystals that formed were collected and recrystallized from ethanol to give compound **1**; yield: 78%; m.p.: 260°C–262°C, R_f: 0.76 (2:3). IR: 3168 (N—H), 2225 (C≡N), 1726 (C=O, ester). ^{1}H NMR (DMSO-d_6): 14.37 (s, 1H, NH), 7.88–7.89 (d, 1H, thiophene-H), 7.36–7.37 (d, 1H, thiophene-H), 7.21–7.22 (d, 1H, thiophene-H), 3.89–4.01 (q, 2H, OCH_2), 2.44 (s, 3H, CH_3), 0.90–0.92 (t, 3H, CH_3, ester). ^{13}C NMR (DMSO-d_6): 178.49, 164.49, 152.61, 147.48, 134.10, 130.27, 118.85, 115.94, 115.86, 114.11, 61.68, 17.95, 13.34. Anal calcd. For $C_{14}H_{12}N_2O_2S_2$: C, 55.24; H, 3.97; N, 9.20. Found: C, 55.11; H, 3.82; N, 9.34.

TABLE 3 Toxicity of the most potent compounds against the MCF-7 and HepG2 cell lines

	MCF-7			HepG2		
Compd.	**%viability**	**IC_{50}**	**Toxicity**	**%viability**	**IC_{50}**	**Toxicity**
3a	99.4926	>100	Non-toxic	98.2062	> 100	Non-toxic
	97.9904			95.902		
	96.4385			91.8477		
	93.852			90.8123		
	92.8969			90.6227		
5	98.5421	41.5	Very toxic	95.0422	33.6	Toxic
	97.7002			93.1786		
	97.3511			92.5809		
	96.7556			91.4557		
	11.3244			10.7595		
6	99.5054	82.8	Harmful	98.0032	17.4	Harmful
	99.3817			91.944		
	97.9802			85.0585		
	96.0223			66.5825		
	43.075			15.0333		
8	96.8825	>100	Non-toxic	97.6961	>100	Non-toxic
	96.5827			96.1327		
	94.4045			95.1728		
	93.0256			91.8815		
	88.8489			91.7992		
22	99.9169	45.4	Very toxic	98.5841	>100	Non-toxic
	99.5015			97.0796		
	95.9493			95.9292		
	94.8899			89.292		
	18.0723			67.8761		

3.1.2 | Synthesis of 2-[3-cyano-5-ethoxycarbonyl-6-methyl-4-(2′-thienyl) pyridin-2-ylthio] acetamide (**2**)

To a suspension of compound **1** (3.04 g, 10 mmol) and anhydrous sodium carbonate (2.33 g, 22 mmol) in ethanol (30 ml), chloroacetamide (0.94 g, 10 mmol) was added. The mixture was heated under reflux for 30 min. The product that formed on cooling was collected and recrystallized from ethanol as white crystals of **2**; Yield: 91%; m.p: 142°C–144°C, R_f: 0.74 (2:3). IR: 3413, 3300, 3176 (NH_2), 2223 (C≡N), 1717 (C═O, ester), 1681 (C═O, amide). ^{1}H NMR (DMSO-d_6): 7.0.89–7.90 (d, 1H, thiophene-H), 7.65 (s, 1H, NH), 7.38 (s, 1H, thiophene-H), 7.22–7.25 (t, 2H: NH and thiophene-H), 4.02 (s, 2H, SCH_2), 4.09–4.14 (q, 2H, OCH_2), 2.54 (s, 3H, CH_3), 1.00–1.03 (t, 3H, CH_3 of ester group). ^{13}C NMR (DMSO-d_6): 168.36, 165.88, 162.60, 158.43, 144.29, 132.85, 130.49, 127.95, 125.15, 114.61, 103.88, 61.82, 34.14, 23.01, 13.46. Anal. calcd. For $C_{16}H_{15}N_3O_3S_2$: C, 53.17; H, 4.18; N, 11.63. Found: C, 53.23; H, 4.22; N, 11.43.

3.1.3 | Synthesis of 3-amino-5-ethoxycarbonyl-6-methyl-4-(2′-thienyl)thieno [2,3-*b*]pyridine-2-carboxamide (**3a**)

Compound **2** (3.61 g, 10 mmol) was suspended in sodium ethoxide solution (0.12 g sodium in 30 ml abs. ethanol) and heated under reflux for 5 min. The solid that formed was collected and recrystallized from ethanol to give canary yellow crystals of **3a**; yield: 95%; m. p.: 250°C–252°C, R_f: 0.84 (2:3). IR (cm^{-1}): 3454, 3418, 3315, 3262, 3169 (2 NH_2), 1715 (C═O, ester), 1655 (C═O, amide). ^{1}H

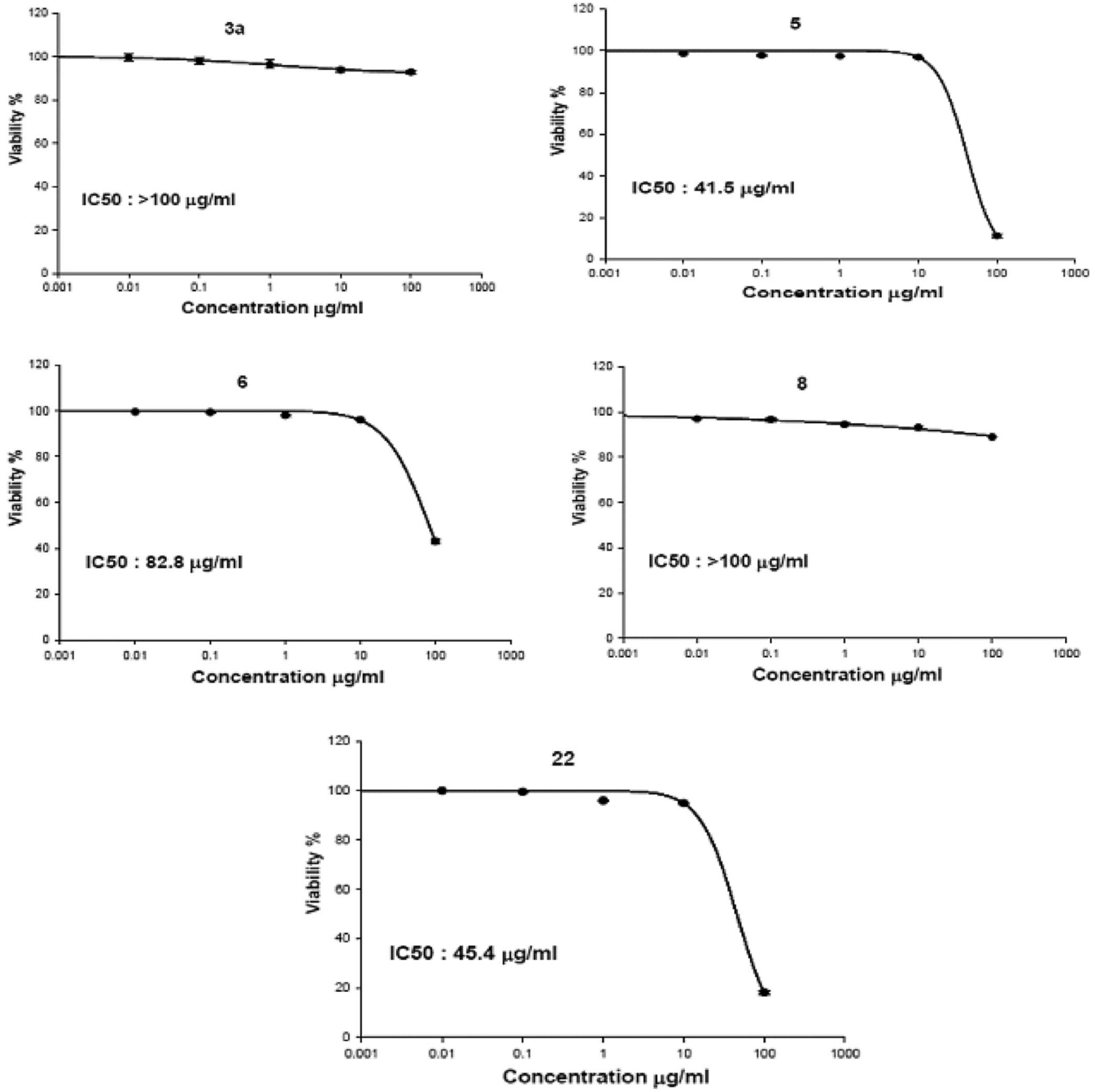


FIGURE 2 The relation between %viability and concentration of the compounds **3a**, **5**, **6**, **8**, and **22** against MCF-7 cell line

NMR (DMSO-d_6): 7.877.88 (d, 1H, thiophene-H), 6.23–7.28 (m, 2H, thiophene-H), 6.96 (s, 2H, $CONH_2$), 5.88 (s, 2H, NH_2 attached to thiophene ring), 4.04–4.08 (q, 2H, OCH_2), 2.57 (s, 3H, CH_3), 0.97–1.00 (t, 3H, CH_3 of ester group). ^{13}C NMR (DMSO-d_6): 166.81, 166.65, 159.01, 154.12, 145.42, 136.62, 132.30, 130.03, 129.36, 127.77, 127.50, 120.76, 98.69, 61.45, 22.64, 13.59. Anal. calcd. For $C_{16}H_{15}N_3O_3S_2$: C, 53.17; H, 4.18; N, 11.63. Found: C, 53.34; H, 4.16; N, 11.50.

3.1.4 | Synthesis of 3-amino-5-ethoxycarbonyl-6-methyl-4(2′-thienyl)thieno [2,3-*b*] pyridine-2-carbonitrile (**3b**)

To a suspension of compound **1** (3.04 g, 10 mmol) and anhydrous sodium carbonate (2.33 g, 22 mmol) in ethanol (30 ml), chloroacetonitrile (0.76 ml, 10 mmol) was added. The resulting mixture was refluxed for 30 min. The precipitate that formed on cooling was collected,

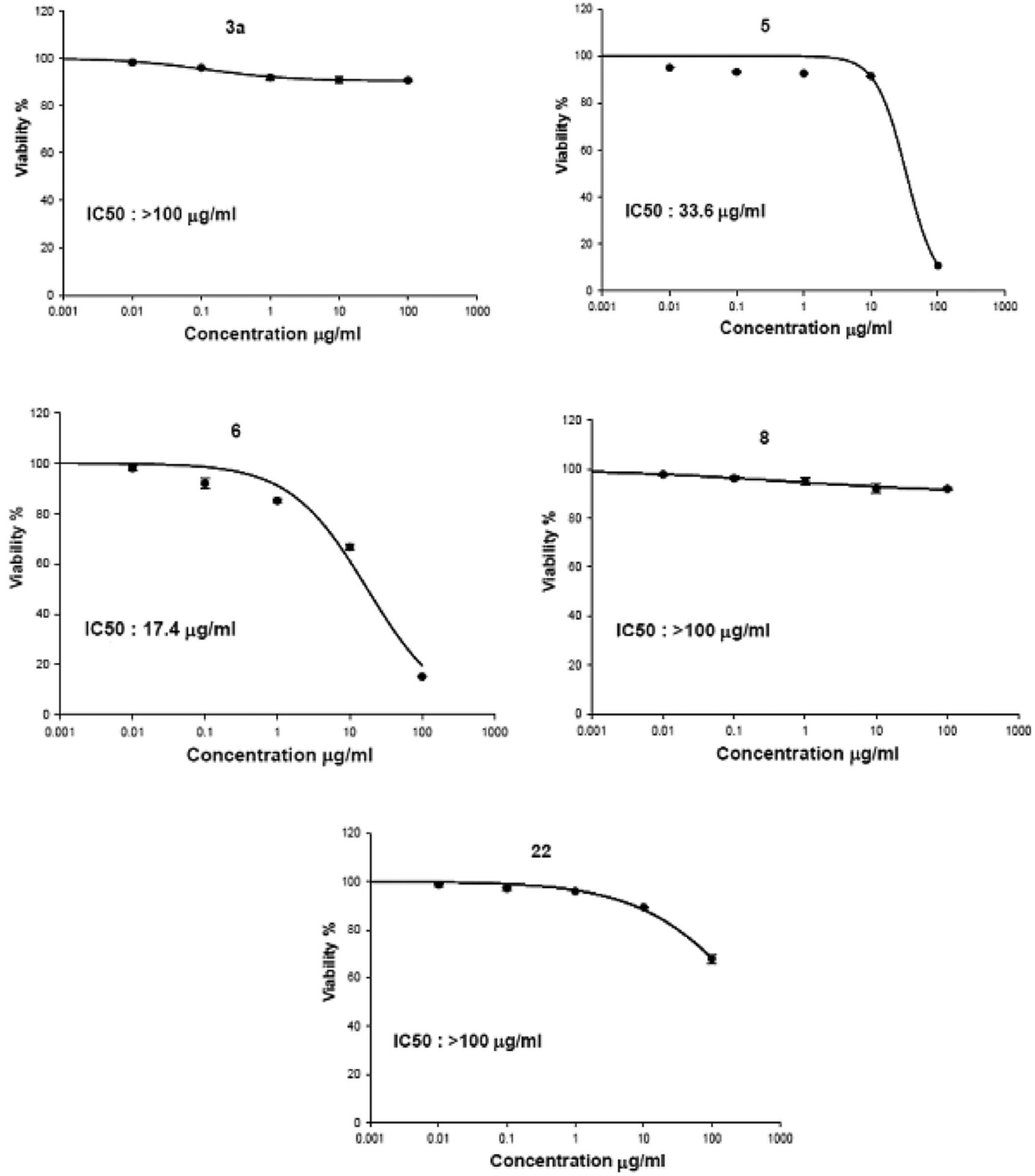


FIGURE 3 The relation between % viability and concentration of the compounds **3a**, **5**, **6**, **8**, and **22** against HepG2 cell line

washed several times with water, and recrystallized from ethanol. Yield: 73%; m.p: 184°C–186°C, R_f: 0.80 (2:3). IR: 3480, 3383, 3125 (NH_2), 2198 (C≡N), 1725 (C═O, ester). ^{1}H NMR ($CDCl_3$): 7.93–7.94 (d, 1H, thiophene-H), 7.28–7.31 (t, 2H, thiophene-H), 5.69 (s, 2H, NH_2), 4.05–4.09 (q, 2H, OCH_2), 2.57 (s, 3H, CH_3), 0.97–1.00 (t, 3H, CH_3 of ester group). Anal. calcd. For $C_{16}H_{13}N_3O_2S_2$: C, 55.96; H, 3.82; N, 12.24. Found: C, 56.09; H, 3.61; N, 12.08.

3.1.5 | Synthesis of 8-ethoxycarbonyl-7-methyl-9-(2′-thienyl)pyrido[3′,2′:4,5]thieno[3,2-*d*] pyrimidine-4-(3*H*)-one (**4**)

A mixture of compound **3a** (3.61 g, 10 mmol) and triethyl orthoformate (2 ml) in acetic anhydride (20 ml) was refluxed for 4 h. The solid that formed was collected and crystallized from ethanol as greenish white needles; yield: 93%; m.p.: >360°C, R_f: 0.92 (3:2). IR: 3165 (N—H), 1724 (C═O, ester), 1662 (C═O, pyrimidineone). ^{1}H NMR ($CDCl_3$): 11.25 (s, 1H, NH); 8.19 (s, 1H, pyrimidine-H), 7.62 (s, 1H, thiophene-H), 7.17–7.22 (m, 2H, thiophene-H), 4.18–4.22 (q, J = 5 Hz, 2H, OCH_2), 2.82 (s, 3H, CH_3), 1.11–1.13 (t, J = 5 Hz, 3H, CH_3 of ester group). ^{13}C NMR ($CDCl_3$): 167.64, 161.78, 160.82, 157.71, 152.63, 145.80, 145.15, 142.60, 133.45, 131.08, 29.59, 127.81, 125.15, 124.03, 63.36, 22.68, 14.34. Anal.calcd. For $C_{17}H_{13}N_3O_3S_2$: C, 54.97; H, 3.53; N, 11.31. Found: C, 54.73; H, 3.50; N, 11.42.

3.1.6 | Synthesis of 4-chloro-8-ethoxycarbonyl-7-methyl-9-(2′-thienyl)pyrido[3′,2′:4,5] thieno[3,2-*d*]pyrimidine (**5**)

Compound **4** (3.71 g, 10 mmol) in phosphorus oxychloride (20 ml) was heated under refluxed on a water bath for 4 h. The cooled reaction mixture was poured with vigorous stirring into ice-water (50 ml). The separated solid was collected and crystallized from ethanol as pale green needles; yield: 98%; m.p.: 184°C–186°C, R_f: 0.77 (3:2). IR: 1731 (C═O, ester). ^{1}H NMR ($CDCl_3$): 8.86 (s, 1H, pyrimidine-H), 7.57–7.59 (d, 1H, thiophene-H), 7.18 (s, 1H, thiophene-H), 7.16 (s, 1H, thiophene-H), 4.15–4.19 (q, J = 7.5 Hz, 2H, OCH_2), 2.78 (s, 3H, CH_3), 1.09–1.12 (t, J = 7.5 Hz, 3H, CH_3 of ester group), ^{13}C NMR ($CDCl_3$): 168.01, 163.89, 159.41, 157.67, 155.20, 154.98, 141.00, 131.15, 130.45, 130.06, 128.90, 127.53, 123.53, 62.36, 24.14, 14.46. Anal. calcd. For $C_{17}H_{12}ClN_3O_2S_2$: C, 52.37; H, 3.10; N, 10.78. Found: C, 52.25; H, 3.14; N, 10.91.

3.1.7 | Synthesis of 8-ethoxycarbonyl-7-methyl-9-(2′-thienyl)pyrido[3′,2′:4,5]thieno[3,2-*d*] pyrimidin-4-(3*H*)-thione (**6**)

Compound **5** (3.89 g, 10 mmol) and thiourea (0.76 g, 10 mmol) in (20 ml) ethanol for 3 h were heated under reflux for 3 h. The precipitate that formed on cooling was collected and then dissolved in 5% sodium hydroxide solution. The resulting solution was filtered and the clear filtrate was acidified with diluted acetic acid whereby a yellow precipitate formed. It was collected and crystallized from ethanol as yellow fine needles; yield: 80%; m.p.: 298°C–300°C, R_f: 0.82 (1:4). IR: 3279 (N—H), 1704 (C═O, ester). ^{1}H NMR (DMSO-d_6): 14.40 (s, 1H, NH); 8.28 (s, 1H, pyrimidine-H), 7.78–7.80 (t, J = 5 Hz, 1H, thiophene-H), 7.15–7.17 (t, J = 5 Hz, 1H, thiophene-H). 4.08–4.12 (q, J = 7 Hz, 2H, OCH_2), 2.63 (s, 3H, CH_3), 1.00–1.03 (t, J = 7 Hz, 3H, CH_3 of ester group). ^{13}C NMR (DMSO-d_6): 185.77, 176.27, 172.43, 165.58, 155.85, 155.67, 148.97, 146.05, 142.56, 139.18, 138.49, 138.11, 136.26, 132.88, 71.03, 32.34, 23.04. Anal. calcd. For $C_{17}H_{13}N_3O_2S_3$: C, 52.69; H, 3.38; N, 10.84. Found: C, 52.86; H, 3.22; N, 10.57.

3.1.8 | Synthesis of 4-substituted 8-ethoxycarbonyl-7-methyl-9-(2′-thienyl)pyrido[3′,2′: 4,5]thieno[3,2-*d*]pyrimidines 7,8; general procedure

To a suspension of compound **6** (3.87 g, 10 mmol) and sodium acetate anhydrous (3.0 g, 22 mmol) in ethanol (40 ml), *N*-(4-tolyl)-2-chloroacetamide or ethyl bromoacetate (10 mmol) was added. The resulting mixture was heated under reflux for 2 h. The precipitate that formed was collected and recrystallized from ethanol to give compounds **7** or **8**, respectively.

*Synthesis of 8-ethoxycarbonyl-7-methyl-9-(2′-thienyl)-4-[N-(4-tolyl)carbamoyl-methylsulfanyl]pyrido[3′,2′:4,5] thieno[3,2-*d*]pyrimidine (**7**)*

It is obtained as a pale green needle by using *N*-(4-tolyl)-2-chloroacetamide; yield: 70%; m.p.: 198°C–200°C, R_f: 0.83 (2:3). IR: 3248 (N—H), 1720 (C═O, ester), 1687 (C═O, anilide). ^{1}H NMR (DMSO-d_6): 9.37 (1H, NH), 8.93 (1H, pyrimidine-H), 7.05–7.59 (m, 7H: Ar—H and thiophene-H), 4.16–4.20 (q, J = 7 Hz, 2H, OCH_2), 4.09 (s, 2H, SCH_2), 2.78 (s, 3H, CH_3), 2.27 (s, 3H, CH_3 of 4-tolyl group), 1.10–1.13 (t, J = 7 Hz, 3H, CH_3 of ester group). ^{13}C NMR (DMSO-d_6): 168.28, 166.92, 163.55, 162.90, 158.95, 154.79, 154.00, 140.71, 135.97, 134.75, 130.44, 130.02, 129.77, 128.87, 128.65, 127.65, 127.42, 123.24, 118.51, 62.66, 34.85, 24.15, 21.56, 14.47. Anal. calcd. For $C_{26}H_{22}N_4O_3S_3$: C, 58.41; H, 4.15; N, 10.48. Found: C, 58.31; H, 4.19; N, 10.60.

*Synthesis of ethyl [8-ethoxycarbonyl-7-methyl-9-(2′-thienyl)pyrido[3′,2′:4,5] thieno[3,2-*d*]pyrimidin-4-ylsulfanyl]acetate (**8**)*

It is obtained as a white needle by using ethyl bromoacetate; yield: 80%; m.p.: 130°C, R_f: 0.68 (1:4). IR (cm^{-1}): 1724 (C═O, ester), 1712 (C═O, ester). ^{1}H NMR (DMSO-d_6): 8.78 (s, 1H, pyrimidine-H), 7.54–7.57 (t, 1H,

thiophene-H), 7.15–7.18 (t, 2H, thiophene-H), 4.15–4.24 (m, 4H, two OCH_2), 4.14 (s, 2H, SCH_2), 2.76 (s, 3H, CH_3), 1.25–1.28 (t, $J = 7$ Hz, 3H, CH_3 of ester group), 1.08–1.11 (t, $J = 7$ Hz, 3H, CH_3 of ester group). Anal. calcd. For $C_{21}H_{19}N_3O_4S_3$: C, 53.26; H, 4.04; N, 8.87. Found: C, 53.51; H, 4.20; N, 8.56.

3.1.9 | Synthesis of 3-[8-ethoxycarbonyl-7-methyl-9-(2′-thienyl)pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-ylsulfanyl]-5-phenyl-2*H*-1,2,4-triazole (**9**)

To a mixture of chloropyrimidine **5** (g, 10 mmol) and using 5-phenyl-3-mercapto-2*H*-1,2,4-triazole in ethanol (30 ml); sodium acetate anhydrous (3.0 g, 22 mmol), was added. The resulting mixture was heated under reflux for 4 h. The precipitate that formed on cooling was collected and recrystallized from ethanol to give a pale green needles of compound **9**; yield: 90%. m.p.: 198°C–200°C, R_f: 0.78 (2:3). IR: 3203 (N—H), 1726 (C═O, ester). ^{1}H NMR (DMSO-d_6): 15.20 (s, 1H, NH), 8.84 (s, 1H, pyrimidine-H), 6.92–8.03 (m, 8H: Ar-H and thiophene-H), 4.08–4.12 (q, $J = 7$ Hz, 2H, OCH_2), 2.64 (s, 3H, CH_3), 0.99–1.02 (t, $J = 7$ Hz, 3H, CH_3 of ester group). ^{13}C NMR (DMSO-d_6): 176.12, 171.60, 167.24, 164.94, 163.88, 163.44, 148.95, 142.53, 141.35, 139.35, 139.06, 138.58, 138.26, 136.74, 136.38, 135.59, 131.24, 129.04, 125.71, 121.97, 70.98, 65.40, 32.39, 28.01, 23.02. Anal. calcd. For $C_{25}H_{18}N_6O_2S_3$: C, 56.59; H, 3.42; N, 15.84. Found: C, 56.33; H, 3.37; N, 15.69.

3.1.10 | Reaction of compound **5** with piperidine or morpholine; synthesis of compound **10** and **11**; general procedure

A mixture of chlorocompound **5** (1.95 g, 5 mmol) and piperidine or morpholine (4 ml) was gently heated under reflux for 3 h. The reaction mixture was triturated with ethanol (10 ml) and then left to cool. The precipitate that formed was collected and recrystallized from ethanol to give compound **10** or **11**, respectively.

*4-Piperidino-8-ethoxycarbonyl-7-methyl-9-(2′-thienyl) pyrido[3′,2′:4,5]thieno [3,2-*d*]pyrimidine (**10**)*

It was obtained as pale yellow white needles; yield: 85%; m.p.: 156°C, R_f: 0.89 (1:4). IR: 1725 (C═O, ester). ^{1}H NMR ($CDCl_3$): δ 8.48 (s, 1H, pyrimidine-H), 7.54–7.55 (s, 1H, thiophene-H), 7.14–7.15 (d, 2H, thiophene-H), 4.11–4.16 (q, 2H, OCH_2), 3.89–3.92 (t, 4H, CH_2NCH_2), 2.73 (s, 3H, CH3), 1.73–1.76 (s, 6H, $CH_2CH_2CH_2$), 1.07–1.10 (t, 3H, CH_3 of ester group). Anal. calcd. For $C_{22}H_{22}N_4O_2S_2$: C, 60.25; H, 5.06; N, 12.78. Found: C, 60.00; H, 5.24; N, 12.53.

*4-Morpholino-8-ethoxycarbonyl-7-methyl-9-(2′-thienyl) pyrido[3′,2′:4,5]thieno [3,2-d]pyrimidine (**11**)*

It was obtained as white needles; yield: 91%; m.p.: 146°C–147°C, R_f: 0.89 (1:4). IR: 1720 (C═O, ester). ^{1}H NMR ($CDCl_3$): 8.49 (s, 1H, pyrimidine-H), 7.54 (s, 1H, thiophene-H), 7.14–7.16 (t, $J = 5$ Hz, 2H, thiophene-H), 4.13–4.17 (q, $J = 7$ Hz, 2H, OCH_2), 3.93–3.95 (t, $J = 5$ Hz, 4H, two OCH_2), 3.85–3.87 (t, $J = 5$ Hz, 4H, two NCH_2), 2.74 (s, 3H, CH_3), 1.08–1.11 (t, $J = 7$ Hz, 3H, CH_3 of ester group). ^{13}C NMR ($CDCl_3$): 168.56, 162.39, 159.11, 157.60, 156.73, 154.83, 139.87, 135.57, 130.09, 129.43, 128.25, 127.22, 123.74, 114.85, 67.40, 62.49, 47.34, 23.93, 14.47. Anal. calcd. For $C_{21}H_{20}N_4O_3S_2$: C, 57.26; H, 4.58; N, 12.72. Found: C, 57.00; H, 4.61; N, 12.48.

3.1.11 | Synthesis of 4-hydrazino-8-ethoxycarbonyl-7-methyl-9-(2′-thienyl)pyrido[3′,2′: 4,5]thieno[3,2-*d*]pyrimidine (**12**)

A mixture of compound 5 (3.89 g, 10 mmol) and hydrazine hydrate (3 ml) in ethanol (20 ml) was heated under reflux for 2 h. The product that formed was collected and recrystallized from ethanol to give white needles of compound **12**; yield: 94%; m.p.:280°C–282°C, R_f: 0.87 (2:3). IR: 3341, 3292, 3227, 3188 ($NHNH_2$), 1729 (C═O, ester), 1644 (C═N). ^{1}H NMR (DMSO-d_6): 9.14 (s, 1H, NH), 8.17 (s, 1H, pyrimidine-H), 7.75–7.76 (d, $J = 5$ Hz, 1H, thiophene-H), 7.10–7.15 (m, 2H, thiophene-H), 4.95 (s, 2H, NH_2), 4.06–4.10 (q, $J = 7$ Hz, 2H, OCH_2), 2.63 (s, 3H, CH_3), 1.00–1.03 (t, $J = 7$ Hz, 3H, CH_3 of ester group). ^{13}C NMR (DMSO-d_6): 176.80, 173.96, 170.26, 164.81, 163.51, 147.34, 143.91, 138.41, 137.60, 137.23, 135.97, 131.44, 120.33, 70.83, 32.24, 23.05. Anal. calcd. For $C_{17}H_{15}N_5O_2S_2$: C, 52.97; H, 3.92; N, 18.17. Found: C, 52.80; H, 3.87; N, 18.35.

3.1.12 | Synthesis of 2-cyano-3-ethoxymethyleneamino-5-ethoxycarbonyl-6-methyl-4-(2′-thienyl)thieno[2,3-b] pyridine (**13**)

A mixture of compound **3b** (3.43 g, 10 mmol), triethyl orthoformate (5 ml) and acetic anhydride (20 ml) was heated under reflux for 4 h. The precipitate that formed after cooling was collected and recrystallized from ethanol to give pale yellow crystals of **13**; yield: 92%, m.p.: 152°C–153°C, R_f: 0.77 (2:3). IR: 2212 (C≡N), 1730 (C═O ester), 1633 (C═N). ^{1}H NMR ($CDCl_3$): 7.63 (s, 1H,

N=CH), 7.44–745 (d, 1H, thiophene-H), 7.04–7.05 (d, $J = 5$ Hz, 1H, thiophene-H), 6.97–6.98 (d, $J = 5$ Hz, 1H, thiophene-H), 4.08–4.12 (q, $J = 7$ Hz, 2H, OCH_2 of ester group), 3.73–3.77 (q, $J = 7$ Hz, 2H, OCH_2 of methanimidate group), 2.68 (s, 3H, CH_3), 1.17–1.20 (t, $J = 7$ Hz, 3H, CH_3 of methanimidate group), 1.04–1.07 (t, $J = 7$ Hz, 3H, CH_3 of ester group). ^{13}C NMR ($CDCl_3$): 167.85, 161.16, 157.44, 156.84, 151.62, 138.67, 134.68, 130.26, 130.02, 127.87, 127.09, 123.66, 118.52, 114.50, 92.99, 63.72, 62.47, 23.77, 14.40. Anal. calcd. For $C_{19}H_{17}N_3O_3S_2$: C, 57.13; H, 4.29; N, 10.52. Found: C, 57.16; H, 4.24; N, 10.37.

3.1.13 | Synthesis of 3-Amino-3,4-dihydro-4-imino-8-ethoxycarbonyl-7-methyl-9-(2′-thienyl) pyrido[3′,2′:4,5]thieno[3,2-*d*] pyrimidine (**14**)

To a suspension of compound **13** (3.99 g, 10 mmol) in Dioxane (10 ml), hydrazine hydrate 99% (2 ml) was added. The reaction mixture was stirred at room temperature for 2 h. The solid that formed was collected and recrystallized from ethanol to give compound **14**; yield: 96%, m.p.: 218°C–220°C, R_f: 0.52 (1:4). IR: 3313, 3282, 3154 (NH_2, NH), 1720 (C=O, ester). ^{1}H NMR (DMSO-d_6): 10.20 (1H, NH), 7.89 (1H, pyrimidine-H), 7.10–7.76 (m, 3H, thiophene-H), 5.74 (s, 2H, NH_2), 4.07–4.11 (q, 2H, OCH_2), 2.61 (s, 3H, CH_3), 1.00–1.03 (t, 3H, CH_3 of ester). ^{13}C NMR (DMSO-d_6): 167.10, 161.58, 153.89, 149.89, 149.70, 144.50, 133.63, 129.39, 128.64, 128.49, 126.52, 123.70, 107.09, 61.40, 55.75, 22.62, 18.52, 13.55. Anal. calcd. For $C_{17}H_{15}N_5O_2S_2$: C, 52.97; H, 3.92; N, 18.17. Found: C, 53.14; H, 3.77; N, 18.00.

3.1.14 | Synthesis of 8-ethoxycarbonyl-9-methyl-7-(2′-thienyl)-[1,2,4]triazolo[4″,3″-*c*] pyrido[3′,2′:4,5]thieno[2,3-*e*]pyrimidine (**15**)

To a mixture of hydrazino compound **12** (0.39 g, 1 mmol) and triethyl orthoformate (5 ml), few drops of acetic anhydride were added. The reaction mixture was heated under reflux for 5 h. The solid product was collected and recrystallized from ethanol as yellowish crystals of compound **15**; yield: 77%; m.p.: 258°C–260°C, R_f: 0.53 (2:3). IR: 1728 (C=O, ester). ^{1}H NMR ($CDCl_3$): 9.00 (s, 1H, pyrimidine-H), 8.85 (s, 1H, triazole-H), 7.54–7.55 (d, 1H, -thiophene-H), 7.14–7.17 (t, 2H, thiophene-H), 4.14–4.18 (q, $J = 7$ Hz, 2H, OCH_2), 2.74 (s, 3H, CH_3), 1.09–1.11 (t, $J = 7$ Hz, 3H, CH_3 of ester group). ^{13}C NMR ($CDCl_3$): 168.28, 162.10, 156.36, 145.88, 141.71, 138.55, 135.39, 134.74, 134.17, 130.22, 129.79, 128.21, 127.27, 123.90, 119.88, 62.43, 23.79, 14.46. Anal. calcd. For $C_{18}H_{13}N_5O_2S_2$: C, 54.67; H, 3.31; N, 17.71. Found: C, 54.45; H, 3.38; N, 17.61.

3.1.15 | Synthesis of 8-ethoxycarbonyl-9-methyl-7-(2′-thienyl)-[1,2,4]triazolo[2″,3″-c] pyrido[3′,2′:4,5]thieno[2,3-e]pyrimidine (**16**)

Method A

A mixture of hydrazino compound **12** (0.38 g, 1 mmol) and formic acid (5 ml) was heated under reflux for 5 h. The solid product was collected and crystallized from ethanol as white crystals of compound **16**; yield: 75%; m.p.: 194°C–196°C, R_f: 0.78 (2:3). IR: 1720 (C=O, ester). ^{1}H NMR ($CDCl_3$): 9.16 (s, 1H, pyrimidine-H), 8.46 (s, 1H, triazole-H), 7.57–7.58 (m, 3H, thiophene-H), 7.18–7.19 (d, 2H, thiophene-H), 4.15–4.19 (q, $J = 7$ Hz, 2H, OCH_2), 2.77 (s, 3H, CH_3), 1.09–1.12 (t, $J = 7$ Hz, 3H, CH_3 of ester group). ^{13}C NMR ($CDCl_3$): 167.74, 162.62, 156.67, 155.92, 148.87, 144.65, 139.13, 137.60, 134.91, 130.35, 129.58, 128.07, 127.29, 124.07, 121.22, 62.60, 23.89, 14.35. Anal. calcd. For $C_{18}H_{13}N_5O_2S_2$: C, 54.67; H, 3.31; N, 17.71. Found: C, 54.82; H, 3.24; N, 17.50.

Method B

A mixture of compound **14** (0.38 g, 1 mmol) and triethyl orthoformate (5 ml) was heated under reflux for 5 h. The solid product was collected and recrystallized from ethanol as white crystals of compound **16**; yield: 70%; m.p.: 194°C–196°C (m.m.p.: 194°C–196°C). The data of FT-IR, 1H-NMR, and ^{13}C-NMR of this product are identical to those reported in method A.

Method C

Compound **15** (0.39 g, 1 mmol) in formic acid (5 ml) was heated under reflux for 5 h. The solid product was collected and crystallized from ethanol as white crystals of compound **16**; yield: 96%; m.p.: 194°C–196°C (m.m.p.: 194°C–196°C). The data of FT-IR, 1H-NMR, and ^{13}C-NMR of this product are identical to those reported in both methods A and B.

3.1.16 | Synthesis of 2-methyl-8-ethoxycarbonyl-9-methyl-7-(2′-thienyl)-[1,2,4] triazolo [2″,3″-*c*]pyrido[3′,2′:4,5]thieno[2,3-*e*] pyrimidine (**18**)

Method A

Hydrazino compound **12** (0.38 g, 1 mmol) in glacial acetic acid (5 ml) was heated under reflux for 5 h. the precipitate that formed on cooling was collected and

recrystallized from ethanol as white crystals of compound **18**; yield: 81%; m.p.: 230°C–232°C, R_f: 0.85 (1:4).. IR: 1718 (C═O, ester). ^{1}H NMR ($CDCl_3$): 9.03 (s, 1H, pyrimidine-H), 7.55–7.56 (d, 1H, thiophene-H), 7.16–7.17 (d, 2H, thiophene-H), 4.14–4.18 (q, 2H, OCH_2), 2.75 (s, 3H, CH_3 attached to pyridine), 2.65 (s, 3H, CH_3 attached to triazole), 1.08–1.11 (t, 3H, CH_3 of ester group). ^{13}C NMR ($CDCl_3$): 168.29, 166.54, 162.60, 156.61, 149.05, 144.68, 139.13, 137.41, 134.96, 130.23, 129.66, 128.28, 127.35, 124.12, 120.11, 62.55, 23.85, 15.47, 14.46. Anal. calcd. For $C_{19}H_{15}N_5O_2S_2$ (409.48): C, 55.73; H, 3.69; N, 17.10. Found: C, 55.45; H, 3.52; N, 17.01.

Method B

A mixture of compound **14** (0.39 g, 1 mmol) and acetic anhydride (5 ml) was heated under reflux for 5 h. The crystalline precipitate that formed on cooling was collected by filtration and recrystallized from ethanol as white crystals of compound **18**; yield: 94%; m.p.: 230°C–232°C (m.m.p.: 230°C–132°C). The data of FT-IR, 1H-NMR, and ^{13}C-NMR of this product are identical to those reported in Method A.

3.1.17 | Synthesis of 4-(1,2,2-triacetylhydrazino)-8-ethoxycarbonyl-7-methyl-9-(2′-thienyl)pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine (**19**)

A mixture of hydrazino compound **12** (0.38 g, 1 mmol) and acetic anhydride (5 ml) was refluxed for 5 h. The precipitate that formed was collected and recrystallized from ethanol as white crystals of compound **20**; yield: 89%; m.p.: 174°C–176°C, R_f: 0.88 (2:3). IR: 1741 (C═O, acetyl), 1714 (C═O, ester). ^{1}H NMR ($CDCl_3$): 8.81 (s, 1H, pyrimidine-H), 7.56–7.58 (d, 1H, thiophene-H), 7.16–7.17 (d, 2H, thiophene-H), 4.14–4.18 (q, $J = 7$ Hz, 2H, OCH_2), 2.76 (s, 3H, CH_3), 2.53 (s, 6H, two $COCH_3$), 2.47 (s, 3H, $COCH_3$), 1.08–1.11 (t, $J = 7$ Hz, 3H, CH_3 of ester group). ^{13}C NMR (DMSO-d_6):): 171.24, 170.28, 167.37, 163.37, 158.62, 153.74, 153.29, 139.77, 134.22, 129.52, 128.83, 127.77, 126.72, 121.79, 120.71, 118.05, 104.93, 61.90, 24.90, 23.89, 23.41, 13.75. Anal. calcd. For $C_{23}H_{21}N_5O_5S_2$: C, 54.00; H, 4.14; N, 13.69. Found: C, 53.71; H, 4.10; N, 13.43.

3.1.18 | Synthesis of 4-(2′-thienyl) methylenehydrazino-8-ethoxycarbonyl-7-methyl-9-(2′-thienyl)pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine (**20**)

A mixture of hydrazino compound **12** (0.38 g, 1 mmol) and thiophene-2-carboxaldehyde (2 ml) was refluxed with ethanol for 3 h. The solid product was collected and recrystallized from ethanol as yellow crystals; yield: 95%; m.p.: 254°C–256°C, R_f: 0.83 (1:4). IR: 3196 (NH), 1726 (C═O, ester). ^{1}H NMR ($CDCl_3$): 10.92 (s, 1H, NH), 8.43 (s, 2H, pyrimidine-H, N═CH), 7.13–7.67 (m, 6H, thiophene-H), 4.17–4.22 (q, $J = 8$ Hz, 2H, OCH_2), 2.79 (s, 3H, CH_3), 1.11–1.14 (t, $J = 8$ Hz, 3H, CH_3 of ester group). Anal. calcd. For $C_{22}H_{17}N_5O_2S_3$: C, 55.10; H, 3.57; N, 14.60. Found: C, 55.23; H, 3.40; N, 14.41.

3.1.19 | Synthesis of 4-(3,5-Dimethylpyrazol-1-yl)-8-ethoxycarbonyl-7-methyl-9-(2′-thienyl) pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidine (**21**)

A mixture of hydrazino compound **12** (0.38 g, 1 mmol) and acetylacetone (10 ml) was gently refluxed for 3 h. the mixture was then mashed with ethanol (15 ml) and left to cool. The solid product was collected and recrystallized from ethanol as buff crystals; yield: 95%; m.p.: 200°C–202°C, R_f: 0.55 (1:4). IR: 1731 (C═O, ester). ^{1}H NMR ($CDCl_3$): 8.78 (s, 1H, pyrimidine-H), 7.57–7.58 (d, $J = 5$ Hz, 1H, thiophene-H), 7.17–7.21 (m, 2H, thiophene-H), 6.06 (s, 1H, pyrazole-H), 4.14–4.18 (q, $J = 7$ Hz, 2H, OCH_2), 2.77 (s, 3H, CH_3), 2.76 (s, 3H, CH_3), 2.36 (s, 3H, CH_3 attached to pyrazole ring), 1.09–1.12 (t, $J = 7$ Hz, 3H, CH_3 of ester group). ^{13}C NMR ($CDCl_3$): 168.61, 167.05, 158.07, 158.09, 154.46, 153.55, 151.95, 144.14, 139.71, 135.59, 129.59, 129.43, 128.19, 127.27, 122.88, 119.83, 111.61, 62.52, 24.07, 16.13, 14.46, 14.39. Anal. calcd. For $C_{22}H_{19}N_5O_2S_2$: C, 58.78; H, 4.26; N, 15.58. Found: C, 58.90; H, 4.15; N, 15.37.

3.1.20 | Reaction of compound 14 with thiophene-2-carboxaldehyde; formation of compounds **20** and **22**

A mixture of compound **14** (0.38 g, 1 mmol) and thiophene-2-carboxaldehyde (2 ml) ethanol (30 ml) was heated under reflux for 3 h. The precipitate that formed on hot was collected and recrystallized from dioxane and identified as 4-(2′-thienyl)methylenehydrazino-8-ethoxycarbonyl-7-methyl-9-(2′-thienyl)pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine (**20**) which is identical to that described above in all aspects; yield: 24%. The filtrate from the above crude compound was allowed to cool whereby a crystalline compound precipitated. It was collected and recrystallized from ethanol to give pale yellow needles of 3-(2′-[thienyl]methylene)amino-3,4-dihydro-4-imino-8-ethoxycarbonyl-7-methyl-9(2′-thienyl) pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine (**22**; yield: 50%; m.p.: 232°C–234°C, R_f: 0.78 (1:4). IR: 3245 (NH), 1726 (C═O, ester). ^{1}H NMR ($CDCl_3$): 10.09 (s, 1H, NH), 9.11 (s, 1H,

pyrimidine-H), 8.50 (s, 1H, CH═N), 7.09–8.03 (m, 6H, thiophene-H), 4.17–4.21 (q, $J = 5$ Hz, 2H, OCH_2), 2.81 (s, 3H, CH_3), 1.13–1.15 (t, $J = 5$ Hz 3H, CH_3 ester). Anal. calcd. For $C_{22}H_{17}N_5O_2S_3$: C, 55.10; H, 3.57; N, 14.60. Found: C, 55.00; H, 3.31; N, 14.77.

3.2 | Biological activity

3.2.1 | Antifungal activity

This study was carried out by using the disk diffusion method, which was reported by Kwon-Chung and Bennett [42], because of the inhibition zone diameters were clear with good definition. The inhibition zones (mm) of the screened compounds were compared with those of Voriconazole, which was used as a reference for the antifungal tests.

3.2.2 | Anticancer activity

The SRB method of monitoring in vitro cytotoxicity was used with multi well plates. The stock concentration of the entire synthesized compounds in DMSO was 10 μg/ml, and this was used to prepare the working dilution. The final DMSO concentration used in the experiments was ≤0.5% as the working concentration. The compounds in serial dilutions (0.01, 0.1, 1.0, 10, and 100 μg/ml) were added after 24 h of culture and the cells were cultured for another 24 h at 37°C. The cell viability was determined in each experiment using MTT colorimetric assay [43,44]. Data were calculated as percent of cell viability.

Cell cultures

MCF7: Breast Adenocarcinoma and HepG2: Hepatocellular carcinoma cell lines were obtained from Nawah Scientific Inc., (Mokatam, Cairo, Egypt). Cells were maintained in DMEM media supplemented with 100 mg/ml of streptomycin, 100 units/ml of penicillin, 10% of heat-inactivated fetal bovine serum in humidified, and 5% (v/v) CO_2 atmosphere at 37°C.

Cytotoxicity assay

Cell viability was assessed by SRB assay. Aliquots of 100 μl cell suspension (5 × 10^3 cells) were in 96-well plates and incubated in complete media for 24 h. Cells were treated with another aliquot of 100 μl media containing drugs at various concentrations ranging from (0.01, 0.1, 1, 10, 100 μg/ml). After 72 h. of drug exposure, cells were fixed by replacing media with 150 μl of 10% TCA and incubated at 4°C for 1 h. The TCA solution was removed, and the cells were washed five times with distilled water. Aliquots of 70 μl SRB solution (0.4% w/v) were added and incubated in a dark place at room temperature for 10 min. Plates were washed three times with 1% acetic acid and allowed to air-dry overnight. Then, 150 μl of TRIS (10 μM) was added to dissolve protein-bound SRB stain; the absorbance was measured at 540 nm using a BMG L ABTECH® -FLUO star Omega microplate reader (Ortenberg, Germany). The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing un reacted control cells to the maximum concentration (0.5%) of DMSO used in each assay.

4 | CONCLUSION

In this paper, we have reported a facile approach for synthesis of new thienylthieno[2.3-*b*]pyridines and thienylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidines started from 3-cyano-5-ethoxcarbonyl-6-methyl-4-(2′-thienyl) pyridine-2-(1*H*)-thione (**1**). In addition, 4-hydrazino-8-ethoxycarbonyl-7-methyl-9-(2′-thienyl)pyrido[3′,2′:4,5] thieno[3,2-*d*]pyrimidine (**12**) and 3-Amino-3,4-dihydro-4-imino-8-ethoxycarbonyl-7-methyl-9-(2′-thienyl)pyrido [3′,2′:4,5]thieno[3,2-d]pyrimidine (**14**) were used as precursors for synthesizing novel thienylpyrido[3′,2′:4,5] thieno[3,2-*d*]pyrimidines as well as their condensed *s*-triazolo derivatives. All synthesized compounds were characterized, elemental and spectral analyses such as IR, ^{1}H NMR, and ^{13}C NMR. In addition, majority of synthesized compounds were screened in vitro for their antifungal activity against five strains of fungi. Moreover, some of the synthesized compounds were screened in vitro for their anticancer activity against HEPG-2 and MCF-7 cell lines. The results obtained, encourage us to publish this research in your esteemed journal, JHC.

DATA AVAILABILITY STATEMENT

Data available in supplementary material of the article.

REFERENCES

[1] N. Murakami, H. Takase, T. Saito, K. Iwata, H. Miura, T. Naruse, *Eur. J. Pharmacol.* **1998**, *352*, 81. https://doi.org/10.1016/s0014-2999(98)00342-2

[2] R. Kulandasamy, A. V. Adhikari, J. P. Stables, *Eur. J. Med. Chem.* **2009**, *44*, 4376. https://doi.org/10.1016/j.ejmech.2009.05.026

[3] X. Lu, B. Wan, S. G. Franzblau, Q. You, *Eur. J. Med. Chem.* **2011**, *46*, 3551. https://doi.org/10.1016/j.ejmech.2011.05.018

[4] N. K. Kaushik, H. S. Kim, Y. J. Chae, Y. N. Lee, G.-C. Kwon, E. H. Choi, I. T. Kim, *Molecules* **2012**, *17*, 11456. https://doi.org/10.3390/molecules171011456

[5] C. K. Ryu, S. K. Lee, J. Y. Han, O. J. Jung, J. Y. Lee, S. H. Jeong, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2617. http://doi.org/10.1016/j.bmcl.2005.03.042
[6] L. J. Gonzalez, C. E. Stephens, T. Wenzler, R. Brun, F. A. Tanious, W. D. Wilson, T. Barszcz, K. A. Werbovetz, D. W. Boykin, *Eur. J. Med. Chem.* **2007**, *42*, 552. https://doi.org/10.1016/j.ejmech.2006.11.006
[7] J. B. Hudson, E. A. Graham, N. Miki, G. H. N. Towers, L. L. Hudson, R. Rossi, A. Carpita, D. Neri, *Chemosphere* **1989**, *19*, 1329. https://doi.org/10.1016/0045-6535(89)90080-5
[8] H. J. Jung, Y. S. Song, C. J. Lim, E. H. Park, *J. Ethanopharmacol.* **2009**, *126*, 355. https://doi.org/10.1016/j.jep.2009.08.031
[9] W. O. Chi, L. H. Chang, *Inorg. Chim. Acta* **1986**, *125*, 203. https://doi.org/10.1016/S0020-1693(00)81212-8
[10] D. C. S. Friedman, P. Friedman, *J. Mol. Struct. THEOCHEM* **1995**, *333*, 71. https://doi.org/10.1016/0166-1280(94)03930-J
[11] M. K. Parai, G. Panda, V. Chaturvedi, Y. K. Manju, S. Sinha, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 289. https://doi.org/10.1016/j.bmcl.2007.10.083
[12] D. Caridha, A. K. Kathcart, D. Jirage, N. C. Waters, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3863. https://doi.org/10.1016/j.bmcl.2010.05.039
[13] G. Fear, S. Komarnytsky, I. Raskin, *Pharm. Therap.* **2007**, *113*, 354. https://doi.org/10.1016/j.pharmthera.2006.09.001
[14] V. C. Gibson, C. Redshaw, G. A. Solan, *Chem. Rev.* **2007**, *107*, 1745. https://doi.org/10.1021/cr068437y
[15] X. Q. Ma, D. R. Gang, *Nat. Prod. Rep.* **2004**, *21*, 752.
[16] C. Vidaillac, J. Guillon, C. Arpin, I. Forfar-Bares, B. B. Ba, J. Grellet, S. Moreau, D. H. Caignard, C. Jarry, C. Quentin, *Antimicrob. Agents Chemother.* **2007**, *51*, 831. https://doi.org/10.1128/aac.01306-05
[17] C. Temple, G. A. Rener, W. R. Waud, P. E. Noker, *J. Med. Chem.* **1992**, *35*, 3686. https://doi.org/10.1021/jm00098a014
[18] P. K. Mahata, C. Venkatesh, U. K. S. Kumar, H. Junjappa, *J. Org. Chem.* **2003**, *68*, 3966. https://doi.org/10.1021/jo034053l
[19] M. Balasubramanian, J. G. Keay, in *Comprehensive Heterocyclic Chemistry II*, Vol. 5 (Eds: A. R. Katritzky, C. W. Rees, E. F. V. Scriven), Pergamon, Oxford **1996**, p. 245.
[20] M. E. Schnute, D. J. Anderson, R. J. Brideau, F. L. Ciske, S. A. Collier, M. M. Cudahy, M. Eggen, M. J. Genin, T. A. Hopkins, T. M. Judge, E. J. Kim, M. L. Knechtel, S. K. Nair, J. A. Nieman, N. L. Oien, A. Scott, S. P. Tanis, V. A. Vaillancourt, M. W. Wathen, J. L. Wieber, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3349.
[21] M. E. Schnute, M. M. Cudahy, R. J. Brideau, F. L. Homa, T. A. Hopkins, M. L. Knechtel, N. L. Oien, T. W. Pitts, R. A. Poorman, M. W. Wathen, J. L. Wieber, *J. Med. Chem.* **2005**, *48*, 5794. https://doi.org/10.1021/jm050162b
[22] R. H. Bahekar, M. R. Jain, P. A. Jadav, V. M. Prajapati, D. N. Patel, A. A. Gupta, A. Sharma, R. Tom, D. Bandyopadhya, H. Modi, P. R. Patel, *Bioorg. Med. Chem.* **2007**, *15*, 6782. https://doi.org/10.1016/j.bmc.2007.08.005
[23] A. E. Abdel-Rahman, E. A. Bakhite, E. A. Al-Taifi, *Pharmazie* **2003**, *58*, 372.
[24] S. M. Z. Eldin, *Naturforsch. B: Chem. Sci.* **1999**, *54b*, 674. https://doi.org/10.1515/znb-1999-0517
[25] A. M. Hussin, F. A. Abu-Shanab, E. A. Ishak, *Phosphorus Sulfur Silicon Relat. Elem.* **2000**, *159*, 55. https://doi.org/10.1080/10426500008043650
[26] I. Hayakawa, R. Shioya, T. Agatsuma, H. Furukawa, Y. Sugano, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3411. https://doi.org/10.1016/j.bmcl.2004.04.079
[27] A. M. R. Bernardino, L. C. da Silva Pinheiro, C. R. Rodrigues, N. I. Loureiro, H. C. Castro, A. Lanfredi-Rangel, J. Sabtini-Lopes, J. C. Borges, J. M. Carvalho, G. A. Romeiro, V. F. Ferreira, I. C. P. P. Fruguphetti, M. A. Vannier-Santos, *Bioorg. Med. Chem.* **2006**, *14*, 5765. https://doi.org/10.1016/j.bmc.2006.03.013
[28] A. Krauze, S. Germame, O. Eberlins, I. Sturms, V. Klusa, G. Duburs, *Eur. J. Med. Chem.* **1999**, *34*, 301. https://doi.org/10.1016/S0223-5234(99)80081-6
[29] S. S. Bundhoo, R. A. Anderson, E. Sagan, J. Dada, R. Harris, J. P. Halcox, D. Lang, P. E. James, *J. Cardiovasc. Pharmacol.* **2011**, *58*, 550. https://doi.org/10.1097/fjc.0b013e31822f578c
[30] R. J. G. Peters, S. R. Mehta, K. A. A. Fox, F. Zhao, B. S. Lewis, S. L. Kopecky, R. Diaz, P. J. Commerford, V. Valentin, S. Yusuf, *Circulation* **2003**, *108*, 1682. https://doi.org/10.1161/01.CIR.0000091201.39590.CB
[31] B. Leal, I. F. Afonso, C. R. Rodrigues, P. A. Abreu, R. Garrett, L. C. S. Pinheiro, A. R. Azevedo, J. C. Borges, P. F. Vegi, C. C. C. Santos, F. C. A. da Silveira, L. M. Cabral, I. C. P. P. Frugulhetti, A. M. R. Bernardino, D. O. Santos, H. C. Castro, *Bioorg. Med. Chem.* **2008**, *16*, 8196. https://doi.org/10.1016/j.bmc.2008.07.035
[32] A. Y. Hassan, M. T. Sarg, M. M. Said, S. A. El-Sebaey, *Univ. Org. Chem.* **2013**, *1*, 2. https://doi.org/10.7243/2053-7670-1-2
[33] H. P. Buchstaller, C. D. Siebert, R. Steinmetz, I. Frank, M. L. Berger, R. Gottschlich, J. Leibrock, M. Krug, D. Steinhilber, C. R. Noe, *J. Med. Chem.* **2006**, *49*, 864. https://doi.org/10.1021/jm0503493
[34] B. C. H. May, J. A. Zorn, J. Witkop, J. Sherrill, A. C. Wallace, G. Legname, S. B. Prusiner, F. E. Cohen, *J. Med. Chem.* **2007**, *50*, 65. https://doi.org/10.1021/jm061045z
[35] V. P. Litvinov, V. V. Dotsenko, S. G. Krivokolysko, *Russ. Chem. Bull.* **2005**, *54*, 864.
[36] E. A.-G. Bakhite, *Phosphorus Sulfur Silicon Relat. Elem.* **2003**, *178*, 929. https://doi.org/10.1080/10426500307855
[37] V. V. Dotsenko, D. S. Buryi, D. Y. Lukina, S. G. Krivokolysko, *Russ. Chem. Bull., Int Ed.* **2020**, *69*, 1829.
[38] S. M. Eldin, N. G. Michael, F. A. Attaby, *Egypt. J. Pharm. Sci.* **1994**, *34*, 805.
[39] E. A. Bakhite, A. E. Abdel-Rahman, O. S. Mohamed, E. A. Thabet, *J. Chem. Res.* **2003**, *2003*, 58. https://doi.org/10.3184/030823503103174956
[40] E. A. Bakhite, A. E. Abdel-Rahman, E. A. Al-Taifi, *J. Chem. Res.* **2005**, *2005*, 147. https://doi.org/10.3184/0308234054213726
[41] M. E. A. Zaki, *Molecules* **1998**, *3*, 71. https://doi.org/10.3390/30300071
[42] K. J. Kwon-Chung, J. E. Bennett, *Medical Mycology*, Lea & Febiger, Philadelphia, PA **1992**.
[43] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst.* **1990**, *82*, 1107. https://doi.org/10.1093/jnci/82.13.1107
[44] R. M. Allam, A. M. Al-Abd, A. Khedr, O. A. Sharaf, S. M. Nofal, A. E. Khalifa, H. A. Mosli, A. B. Abdel-Naim,

Toxicol. Lett. **2018**, *291*, 77. https://doi.org/10.1016/j.toxlet.2018.04.008

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: S. Abuelhassan, E. A.-G. Bakhite, A. E. Abdel-Rahman, A. F. M. El-Mahdy, *J Heterocyclic Chem* **2021**, 1. https://doi.org/10.1002/jhet.4310