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Synthesis, Reactions, and Antimicrobial Activity of Novel Heterocyclic Compounds Containing Cyclopenta[*d*]thieno[2,3-*b*]pyridine Moiety and Related Fused Heterocycles

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Abstract—We describe a simple method to synthesize novel 4-cyano-1-morpholin-4-yl-6,7-dihydro-5*H*-cyclopenta[*c*]pyridine-3-thione by the reaction of 3-amino-1-thioxo-1,5,6,7-tetrahydrocyclopenta[*c*]thiopyran-4-carbonitrile with morpholine through Dimroth rearrangement. The 1-amino-5-morpholin-4-yl-7,8-dihydro-6*H*-cyclopenta[*d*]thieno[2,3-*b*]pyridine-2-carboxamide, which was synthesized by two methods, was used as a versatile precursor for synthesis of new thienopyrimidines fused to cyclopenta[*d*]pyridine ring system. Consequently, reaction of the amino-carboxamide with diethylmalonate, triethyl orthoformate, and cycloalkanones afforded the corresponding fused pyrimidine heterocycles. On the other hand, chloroacetylation of the amino-carboxamide using chloroacetyl chloride in dioxane produced the chloroacetyl amino derivative that in turn underwent cyclocondensation upon reaction with acetic anhydride to give the chloromethyl pyrimidinone. The latter compound was subjected to react with various nitrogen nucleophiles by nucleophilic substitution reactions to yield the akyl (aryl)amino methyl pyrimidinones. Subsequently, treatment of the phenyl aminomethyl derivative with formaldehyde under Mannich conditions produced the imidazopyridothienopyrimidine ring system. Similarly, reaction of the phenyl aminomethyl pyrimidonone with chloroacetyl chloride afforded a new heterocyclic system namely, cyclopentapyridothienopyrimidopyrazine. On the other hand, reaction of the amino-carboxamide with carbon disulfide in pyridine led to the formation of oxopyrimidine thione, which was alkylated by the reaction with different α-halo carbonyl compounds. The newly synthesized compounds were fully characterized on the basis of elemental and spectral analyses. Alternatively, some of these compounds revealed promising antibacterial and antifungal activities through the in vitro screening.

Keywords: cyclopentathienopyridine, pyrimidine, pyrazine, imidazole, synthesis, antimicrobial activity

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INTRODUCTION

Cyclopentapyridine was considered as the main structure of many naturally occurring monoterpenoid alkaloids (Fig. 1), namely, (–)-plectrodorine and (+)-oxerin [1, 2], tecomanine [3], anti-androgenic and anti-cancer alkaloids louisianins A–D [4, 5], skytanthine [6, 7], and actinidine [8]. Also, thienopyridines hold a prominent place in heterocyclic chemistry and revealed broad spectrum of biological activities, such as antibacterial [9], antifungal [10], anti-inflammatory [11], antimicrobial [12], neurotropic activities [13], and immunostimulating [14] activities.

On the other hand, thienopyrimidine derivatives are widely used for many applications in medicinal chemistry. Certain thienopyrimidines exhibit antiviral [15, 16], antimicrobial [17], antiallergic [18], antidepressant [19], antihistamionic [20], and antitumor activities [21, 22]. In the light of the biological importance of cyclopentapyridines, thienopyridines, and thienopyrimidines and in continuation of our program for synthesis of novel heterocycles fused to thienocyclopentapyridines and thienotetrahydroisoquinoline ring system [23–27], we have synthesized a series of novel cyclopentathienopyrimidines. Literature survey revealed that the cyclopentapyridothienopyrimidines have not been previously synthesized. As a result of resistance of most of bacterial and fungal strains to the current antimicrobial therapy, we are interested in synthesis of new effective agents. Therefore, the suspected promising biological activities of the cyclopentapyridine and thienopyridine promoted us to study the in vitro antibacterial and antifungal activities of some cyclopentathienopyridine heterocycles in comparison with the standard drugs. Consequently, compounds (**II**), (**III**), (**IV**), (**V**), (**IX**), (**X**), and (**XIa**) were screened for their antimicrobial activity against four strains of bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aruginose*, and *Escherichia coli*) and four strains of fungi (*Geotrichum candidium*, *Candida albicans*, *Trichophyton rubrum*, and *Aspergillus flavus*).

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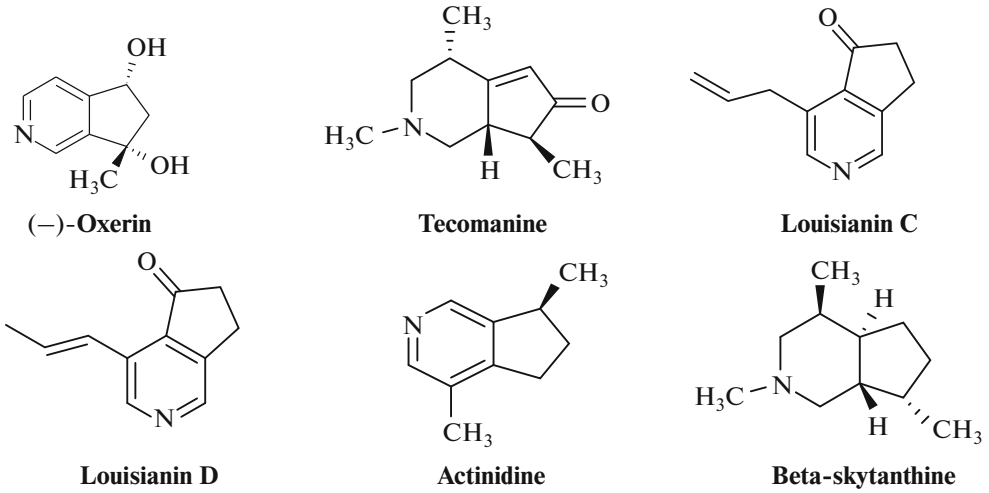


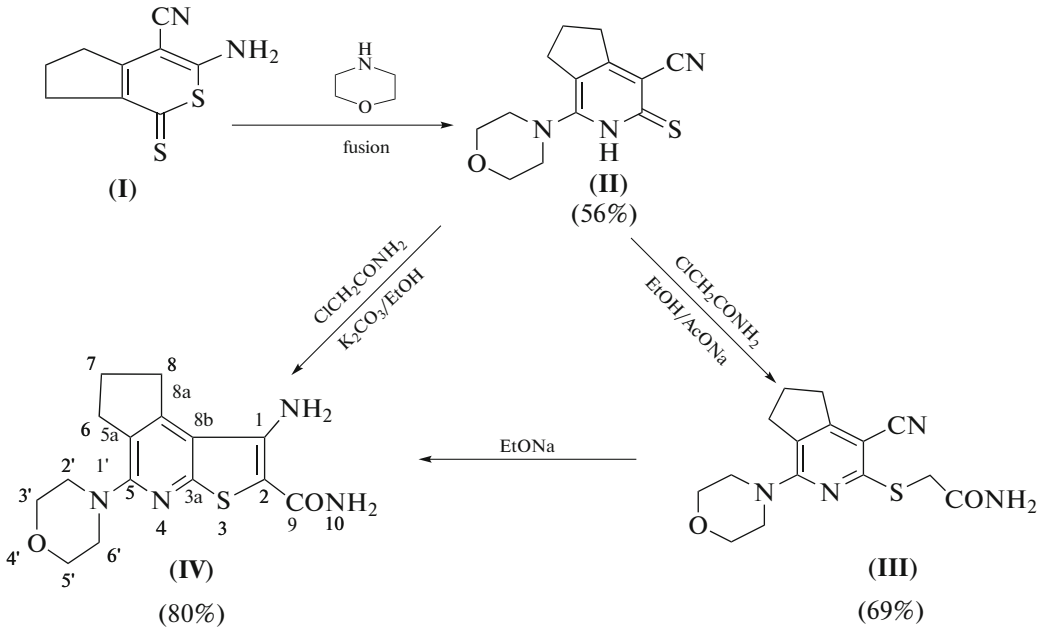
Fig. 1. Chemical structures of terpenoid alkaloids comprising the cyclopentapyridine moiety.

Most of the cyclopentapyridothienopyrimidines revealed promising antibacterial and antifungal activities.

RESULTS AND DISCUSSION

Synthesis of 3-amino-1-thioxo-1,5,6,7-tetrahydrocyclopenta[*c*]thiopyran-4-carbonitrile (**I**) and its transformation into cyclopentathienopyridine derivatives using alkali have been performed previously according to the reported procedure [28]. Herein, we report this transformation using morpholine to produce 3-mercapto-1-morpholin-4-yl-6,7-dihydro-5*H*-cyclopenta[*c*]pyridine-4-carbonitrile (**II**). Compound (**II**) was used as a key intermediate for synthesis of thienocyclopentapyridine and cyclopentapyridothienopyrimidine heterocycles.

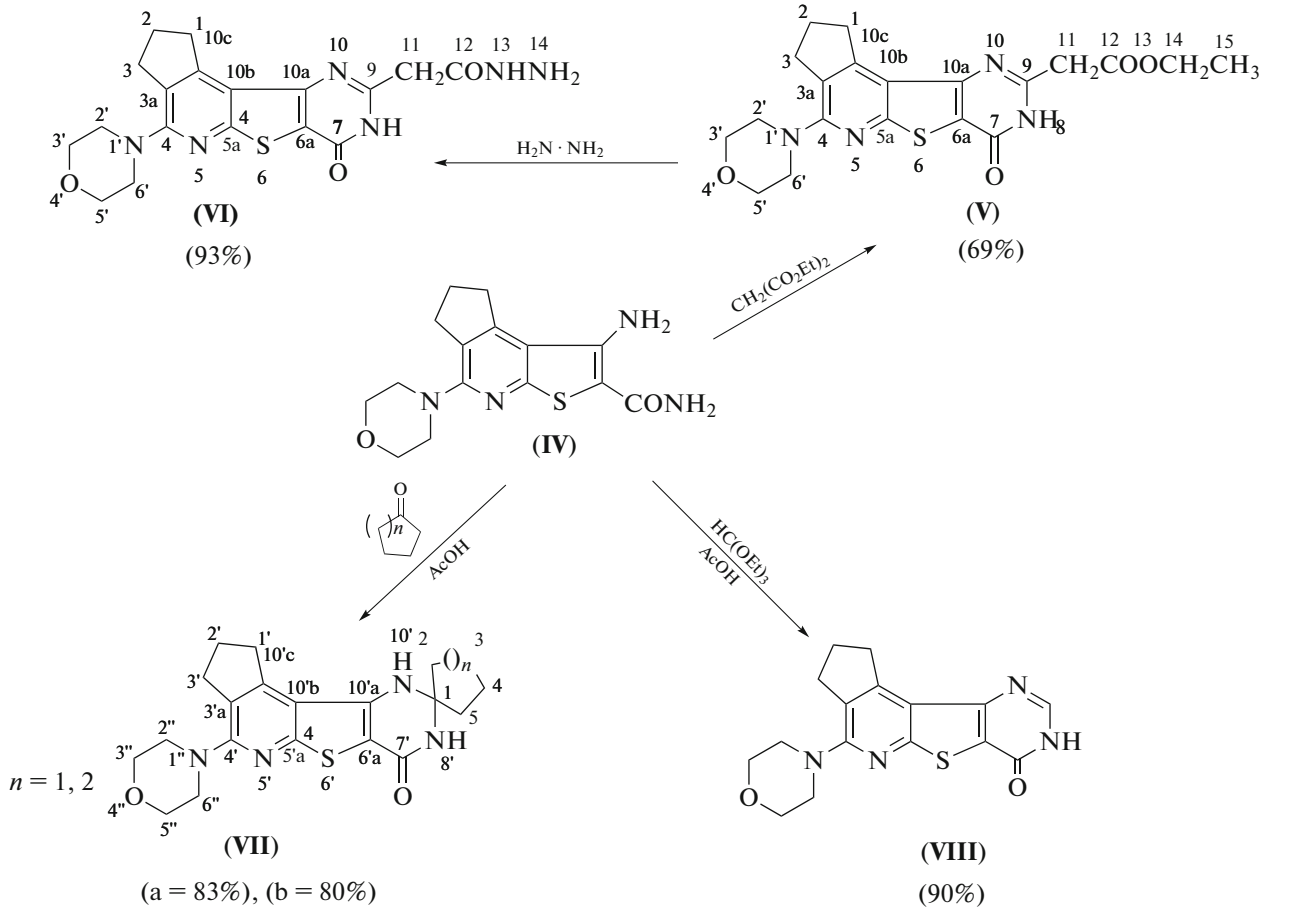
In the present work, we synthesized the novel 1-amino-5-(morpholin-4-yl)-7,8-dihydro-6*H*-cyclopenta[*c*]thieno[2,3-*b*]pyridine-2-carboxamide (**IV**) via two methods (Scheme 1). When the cyclopentapyridine thione (**II**) was subjected to react with chloroacetamide in ethanol and fused sodium acetate, the sulfanyl acetamide (**III**) was obtained. Compound (**III**) underwent Thorpe–Zeigler cyclization upon treatment with ethanolic sodium ethoxide solution to afford the target aminocyclopentathienopyridine carboxamide (**IV**) in a quantitative yield.



Scheme 1. Synthesis of 1-amino-5-morpholin-4-yl-7,8-dihydro-6*H*-cyclopenta[*d*]thieno[2,3-*b*]pyridine-2-carboxamide (**IV**) by two methods.

The latter compound (**IV**) was gained directly via an alternative route through the reaction of pyridinethione (**II**) with chloroacetamide in ethanol and anhydrous potassium carbonate. The solid products obtained by the two pathways were identical in all aspects and compound (**IV**) was elucidated by elemental and spectral data. FT-IR revealed disappearance of absorption band at 2203 cm^{-1} for CN group in compound (**III**) and appearance of two bands characteristic of NH_2 group. ^1H NMR displayed singlet signal at δ 6.62 ppm instead of a signal at δ 3.81 ppm for SCH_2 group in the starting material. ^{13}C NMR exhibited signal at δ 172.59 ppm unique for CO of the amidic group. Also, mass spectrum of the amino-carboxamide (**IV**) showed a molecular ion peak at 318.18.

The amino-carboxamide (**IV**) was used as a versatile synthon starting material for synthesis of new heterocycles fused to the thienopyridine ring system (Scheme 2). Cyclocondensation of (**IV**) with diethyl malonate under solvent-free conditions furnished the ethyl pyrimidinyl acetate (**V**).



Scheme 2. Cyclocondensation reactions of the amino carboxamide (**IV**) with diethylmalonate, cycloalkanones, and triethylorthoformate producing the corresponding pyrimidines (**V**)–(**VIII**).

Hydrazinolysis of the ester group in (**V**) with hydrazine hydrate led to the formation of the corresponding carbohydrazide (**VI**) in an excellent yield. FT-IR spectrum of (**VI**) represented absence of band for CO ester group at 1741 cm^{-1} and presence of absorption bands at 3447, 3314, and 3135 cm^{-1} attributed to $NHNH_2$ in addition to band at 1648 cm^{-1} characteristic of CO group. ^1H NMR displayed singlet signals at δ 6.60 and 6.84 ppm for NH and NH_2 instead of triplet and quartet signals of ester group in compound (**V**). ^{13}C NMR spectrum showed signals at 158.1 and 167.8 ppm for CO pyrimidine and CO carbohydrazide, respectively. Furthermore, reaction of (**IV**) with cycloalkanone and triethyl orthoformate produced the spiro cycloalkanopyrimidinone (**VIIa**,**b**) and the pyrimidinone (**VIII**). Assignment the chemical structures for the newly synthesized pyrimidinone compounds (**VII**) and (**VIII**) was confirmed from their elemental and spectral analyses. FT-IR of (**VIII**) exhibited disappearance of band characteristic of NH_2 and $CONH_2$ in (**IV**) and appearance of broad NH band at 3445 cm^{-1} instead. ^1H

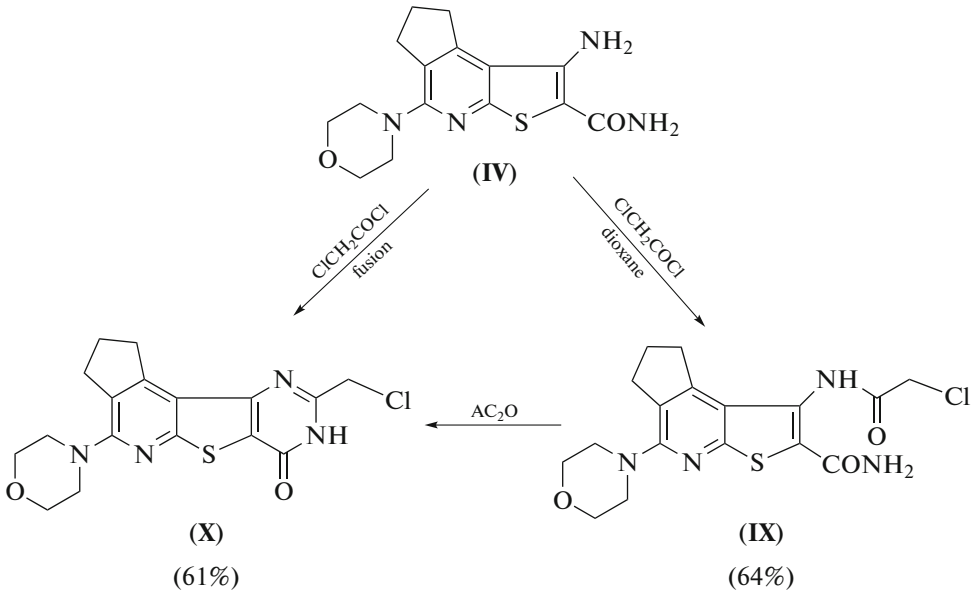
Table 1. Antibacterial activity (inhibition zone and MIC) of compounds **(II)**–**(III)**, **(IV)**, **(V)**, **(IX)**, **(X)**, and **(XIa)**

Bacteria	Samples							Reference
	inhibition zone, mm (MIC, μg/mL)							
	(II)	**(III)**	**(IV)**	**(V)**	**(IX)**	**(X)**	**(XIa)**	ofloxacin
Bacillus cereus (gram-positive)	13(12)	14(11)	9(14)	14(8.0)	13(10)	11[a](10)[b]	18(9.0)	26(3.0)
Staphylococcus aureus (gram-positive)	–	16(9.0)	–	17(8.0)	13(8.0)	13(8.0)	–	28(3.0)
Pseudomonas aruginose (gram-negative)	13(9.0)	9(11)	11(10)	16(9.0)	18(9.0)	15(10)	14(9.0)	22(3.0)
Esherichia coli (gram-negative)	15(9.0)	16(7.0)	12(8.0)	–	–	11(8.0)	14(7.0)	29(3.0)

–, no activity.

NMR displayed singlet signals at δ 8.24, 12.60 ppm attributed to CH and NH in pyrimidine, respectively.

Chloroacetylation of the amino-carboxamide **(IV)** with chloroacetyl chloride in dioxane afforded the chloroacetyl amino derivative **(IX)**, which underwent cyclocondensation reaction through loss of H_2O upon reflux in acetic anhydride to produce the chloromethyl pyrimidinone **(X)** (Scheme 3). The chemical structure of the pyrimidinone derivative **(X)** was confirmed by FT-IR and ^{1}H NMR spectral analyses. FT-IR spectrum revealed disappearance of absorption bands at 3450, 3320, and 3149 cm^{-1} due to NH and NH_2 groups and at 1651 cm^{-1} for CO group in compound **(IX)** and appearance of bands at 3438 and 1655 cm^{-1} particular for NH and CO groups of pyrimidinone. ^{1}H NMR spectrum of compound **(X)** in DMSO-d_6 showed absence of singlet signals at δ 6.62 and 6.91 ppm for NH and NH_2 groups in the chloroacetylamino derivative **(IX)** and presence of singlet signal at δ 12.21 ppm attributed to NH pyrimidine. The latter compound was used as a starting intermediate for synthesis of new heterocycles fused to cyclopentapyridothienopyrimidine moiety.



Scheme 3. Chloroacetylation reaction of the amino carboxamide **(IV)** to give the chloromethyl pyrimidinone **(X)**.

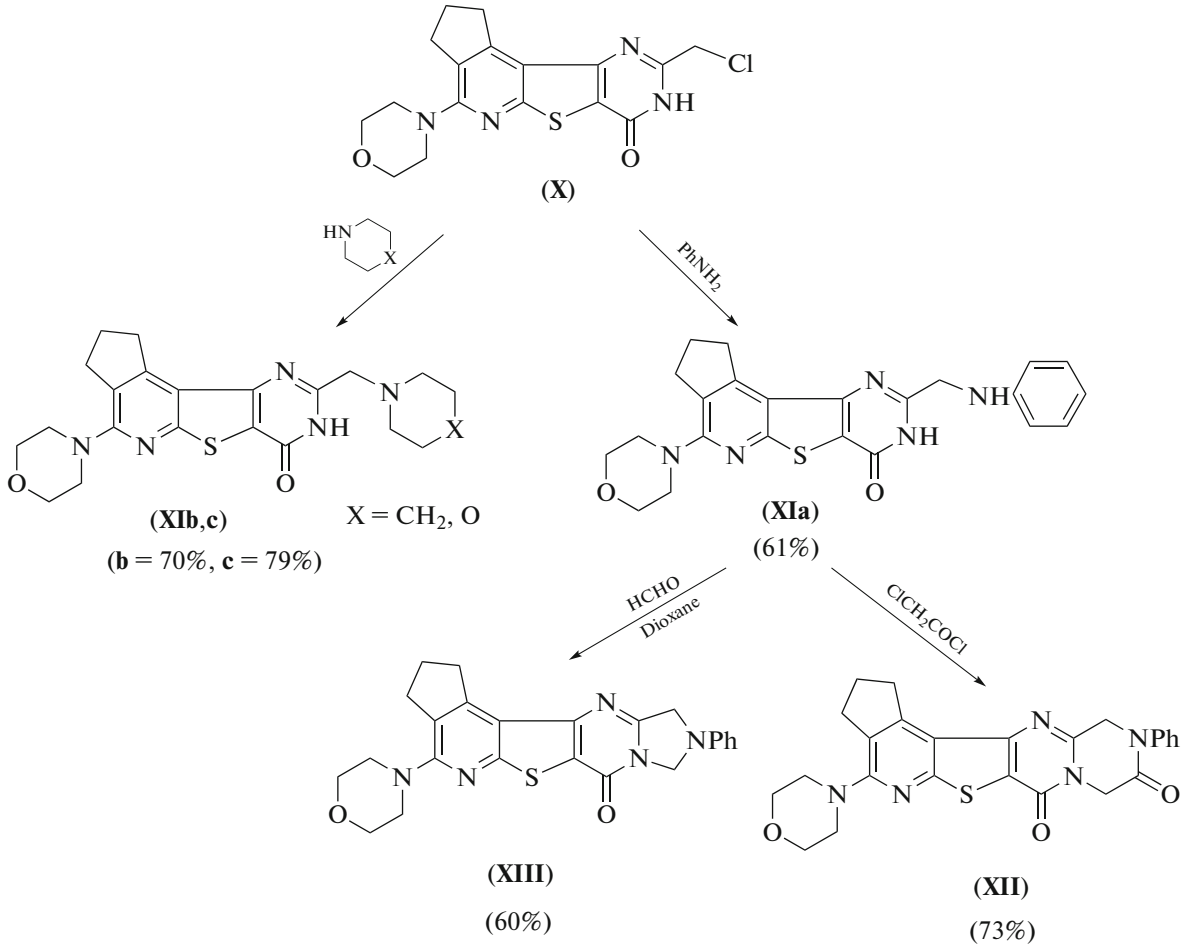
Consequently, the chloride ion in compound **(X)** underwent nucleophilic substitution reactions with various nitrogen nucleophiles to afford the alkyl (aryl)aminomethyl pyrimidinones **(XIa–c)** (Scheme 4). Formation of compounds **(XIa–c)** was established by FT-IR and ^{1}H NMR spectra. FT-IR spectrum of the phenyl aminopyrimidinone **(XIa)** showed absorption band at 3318 cm^{-1} for 2NH and at 1643 cm^{-1} for CO amidic group. ^{1}H NMR in DMSO-d_6 displayed singlet signals at δ 4.32, 6.06, and 12.55 ppm characteristic of CH_2, NHPh, and NH pyrimidine. Treatment of **(XI)** with chloroacetyl chloride and formaldehyde under Mannich conditions afforded the novel heterocyclic sys-

Table 2. Antifungal activity (inhibition zone and MIC) of compounds **(II)**–**(V)**, **(IX)**, **(X)**, and **(XIa)**

Fungi	Samples							
	Inhibition zone, mm (MIC, μg/mL)							
	(II)	**(III)**	**(IV)**	**(V)**	**(IX)**	**(X)**	**(XIa)**	clotrimazole
Geotrichum candidium	15(8.0)	17(10)	15(9.0)	17(7.0)	14(8.0)	17(8.0)	16(7.0)	22(5.0)
Candida albicans	21(8.0)	–	19(7.0)	17(7.0)	20(6.0)	19(7.0)	–	26(3.0)
Trichophyton rubrum	17(8.0)	13(9.0)	15(10)	19(8.0)	–	18(10)	15(12)	25(6.0)
Aspergillus flavus	13(9.0)	16(9.0)	13(10)	16(7.0)	15(9.0)	14(10)	15(9.0)	21(4.0)

–, no activity.

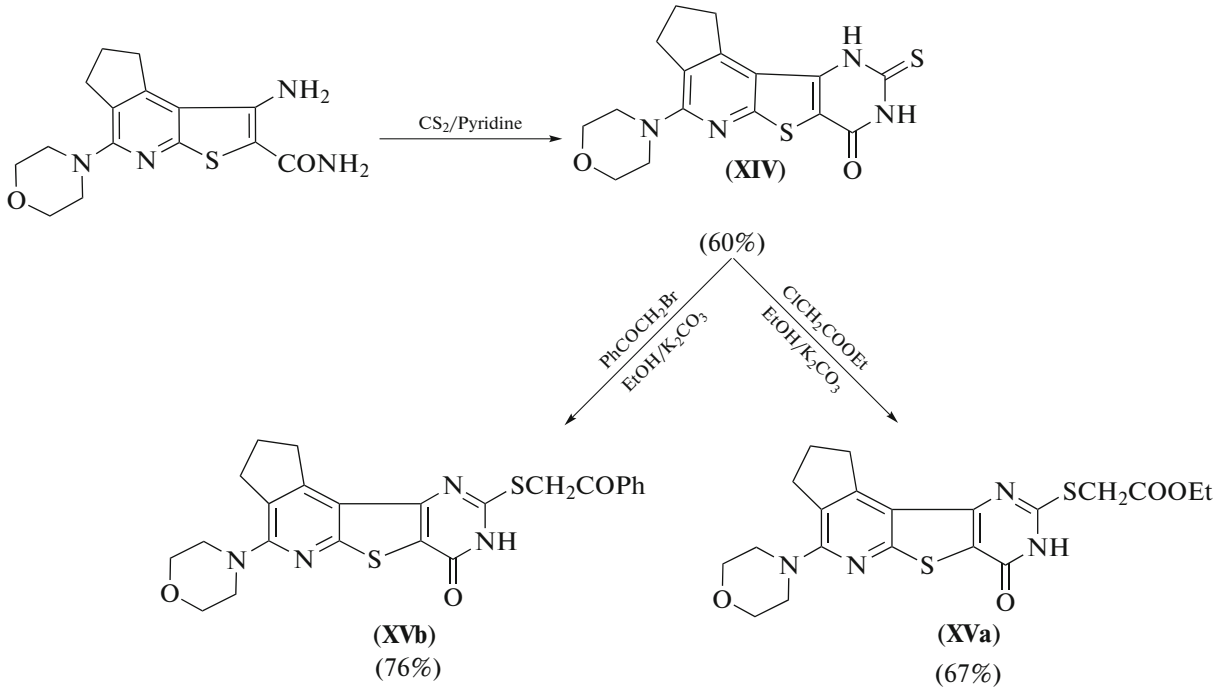
tems, namely, cyclopentapyridothienopyrimidopyrazine **(XII)** and imidazocyclopentapyridothienopyrimidinone **(XIII)**, respectively. Both elemental analysis and spectral data of compounds **(XII)** and **(XIII)** were consistent with the assigned structures. IR spectrum of the pyrazinone compound **(XII)** displayed disappearance of absorption bands at 3318 cm^{-1} in compound **(XIa)** and appearance of bands at 1684 and 1647 cm^{-1} for CO of pyrimidine and CO of pyrazine.^{1}H NMR in DMSO-d_6 exhibited absence of two singlet signals characteristic of 2NH groups and presence of two singlet signals at δ 4.15, 4.84 ppm for $2CH_2$ groups of pyrazine.



Scheme 4. Nucleophilic substitution reactions of **(X)** with various nitrogen nucleophiles.

Subsequently, heating the amino-carboxamide **(IV)** with carbon disulfide in pyridine on a steam bath yielded the oxopyrimidine thione compound **(XIV)**. IR spectrum of compound **(XIV)** revealed appearance of

bands at 3490 and 3369 cm^{-1} for 2NH groups, 1656 cm^{-1} for CONH, and at 1239 cm^{-1} for C=S group. ^1H NMR spectrum in DMSO-d_6 exhibited two singlet signals at δ 11.72 and 12.60 ppm attributed to 2NH groups. Alkylation of the thione group in compound **(XIV)** using α-halocarbonyl compounds, namely, ethylchloroacetate and phenacyl bromide, in ethanol and sodium acetate produced the S-alkylated products **(XVa**,**b)** (Scheme 5). The structure assignment of compounds **(XVa**,**b)** was carried out by IR and ^1H NMR spectral analyses. IR spectrum of **(XVa)** showed absorption bands at 3454 cm^{-1} for NH and at 1725 and 1669 cm^{-1} characteristic of CO ester and CO pyrimidine. ^1H NMR spectrum in DMSO-d_6 displayed triplet and quartet signals at δ 1.20 and 4.17 ppm for the ethyl ester group and singlet signal at δ 13.02 ppm characteristic of pyrimidine NH group.



Scheme 5. Synthesis and alkylation reactions of cyclopentapyridothienopyrimidine thione **(XIV)** affording the S-alkylated products **(XVa,b)**.

Biological Activity

Antibacterial activity of compounds **(II)**, **(III)**, **(IV)**, **(V)**, **(IX)**, **(X)**, and **(XIa)** was determined by measuring the average diameter of the inhibition zones, expressed in mm, and the minimum inhibitory concentration (MIC) is reported in μg/mL. The obtained results are shown in Table 1. All the tested compounds exhibited significant antibacterial activity. The sulfanyl acetamide **(III)** and the chloromethyl pyrimidinone showed the highest antibacterial activity against all tested strains of gram-positive and gram-negative bacteria. The starting cyanocyclopentapyridinethione intermediate **(II)** revealed high antibacterial effect against *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Alkylation of the thione group to give compound **(III)** increased the antibacterial activity especially against *Staphylococcus aureus* with low MIC value. Ring closure of sulfanyl acetamide **(III)** to afford the amino carboxamide **(IV)** decreased the activity against gram-positive and *E. coli* (gram-negative) bacterial strains with slight increasing in activity against *P. aeruginosa*. The chloroacetyl amino derivative **(IX)** displayed moderate activity against gram-positive and *P. aeruginosa* strains, while *E. coli* was resistant to compound **(IX)**. Cyclocondensation of **(IX)** to yield the chloromethyl pyrimidinone **(X)** increased the effect against *E. coli* with showing nearly the same effect against the rest of strains. Nucleophilic substitution of the chloride ion with aniline in compound **(XIa)** strongly affected the antibacterial activity against gram-negative bacteria and *Bacillus cereus* (gram-positive) strains with lowering MIC value to be close to the reference antibiotic (ofloxacin), but the *S. aureus* strain was resistant to **(XIa)**. The ester group in the ethyl oxopyrimidine acetate **(V)** revealed activity against gram-positive bacteria and *P. aeruginosa* with no activity against *E. coli*. The bactericidal effect of ofloxacin works through inhibiting DNA gyrase topoisomerase, types II and IV [29], which is an enzyme necessary to separate DNA being replicated in bacteria, thereby inhibiting bacterial cell division. The power of antibacterial effect of com-

pounds (**III**), (**X**), and (**XIa**) may be due to release of free radicals or ions resulted from presence of cyano and carboxamide groups in compound (**III**), presence of the chloride ion (resembles the fluoride ion in ofolxacin), and existence of NH_2 groups in compound (**XIa**).

Antifungal activity of the examined compounds is reported as zone of inhibition in Table 2. All tested compounds revealed excellent antifungal activities against all fungal strains. Furthermore, the ethylthienocyclopentapyridopyrimidine acetate (**V**) presented the highest antifungal activities against all the tested strains of fungi with the lowest MIC. The cyclopentapyridine thione (**II**) showed high activity with low MIC against the tested fungal strains. Alkylation of (**II**) with chloroactamide decreased the antifungal effect against *Trichophyton rubrum* while showing the same activity as compound (**II**) against *Geotrichum candidium* and *Aspergillus flavus.* At the same time, *Candida albicans* was resistant to compound (**III**). Cyclization of (**III**) to afford the aminothienocyclopentapyridine carboxamide (**IV**) increased the activity against *C. albicans* and exhibited significant activity towards the other fungal strains. The chloride ion in the chloroacetyl amino derivative (**IX**) showed the highest activity against *C. albicans* with moderate activity towards *G. candidium* and *A. flavus.* At the same time *T. rubrum* was resistant to compound (**IX**). On the other hand, ring closure of compound (**IX**) to produce the chloromethyl pyrimidinone (**X**) highly affected the activity against *T. rubrum* with showing comparable activity against the other strains of fungi. Moreover, the phenyl aminomethyl pyrimidinone displayed high activity against *G. candidium* and *A. flavus* with moderate activity against *T. rubrum* and no activity against *C. albicans*.

Clotrimazole works by inhibiting the growth of individual Candida or fungal cells by altering the permeability of the fungal cell wall. It binds to phospholipids in the cell membrane and inhibits the biosynthesis of ergosterol and other sterols required for cell membrane production [30]. The strength of antifungal activity of compounds (**IX**) and (**X**) may be due to existence of chloride ions, which is similar to the clortrimazole.

EXPERIMENTAL

All melting points are uncorrected and measured on a Fisher–John apparatus. Elemental analyses were carried out at the Micro Analytical Center of Chemistry Department, Assiut University. Their results were found to be in good agreement (±0.2%) with the calculated values. FT-IR spectral analyses (v, cm^{-1}) were recorded using potassium bromide disks on a FT-IR 820/PC (Shimadzu). ^1H NMR and ^{13}C spectra were obtained on a Bruker (^1H NMR: 400 MHz, ^{13}C NMR: 100 MHz) and Varian Mercury VX-300 NMR (^1H NMR: 300MHz, ^{13}C NMR: 75 MHz) spectrometers in $CDCl_3$ and DMSO-d_6 using tetramethyl silane (TMS) as an internal standard (chemical shifts are expressed in ppm). All the reactions were monitored by thin layer chromatography (TLC) technique on silica gel coated on aluminum sheets (Silica Gel 60F254 Merck) using UV light. All reactions were carried out under an air atmosphere. Mass spectra were obtained on an ISQ 7000 (70 eV) apparatus at Chemistry Department Lab, Faculty of Science. Compound (**I**) was prepared according to literature procedure [28].

1-Morpholin-4-yl-3-thioxo-3,5,6,7-tetrahydro-2*H*-cyclopenta[*c*]pyridine-4-carbonitrile (II). A solution of the 3-amino-1-thioxo-1,5,6,7-tetrahydrocyclopenta[*c*]thiopyran-4-carbonitrile (**I**) (10.00 g, 0.05 mol) and morpholine (20.00 mL, 0.23 mol) was refluxed in absence of solvent for 1.5 h or until H_2S gas was ceased. The solid precipitate that formed on cooling was filtered off, dried, and recrystallized from ethanol as brilliant pale orange crystals in 56% (7.0 g) yield, mp 220–222°C. FT-IR: 3439 (NH), 2203 (CN), and 1240 (C=S). ^1H NMR (300 MHz, $CDCl_3$): 2.15 (m, 2H, CH_2: C6 cyclopenteno), 2.94 (m, 4H, $2CH_2$: C5 and C7 cyclopenteno), 3.64 (m, 4H, $2CH_2$: $(CH_2)_2N$ morpholinyl), 3.77 (m, 4H, $2CH_2$: $(CH_2)_2O$ morpholinyl), 12.76 (s, 1H, NH). Anal. calcd. for $C_{13}H_{15}N_3OS$ (261.34) C, 59.75; H, 5.79; N, 16.08; S, 12.27%. Found C, 59.62; H, 5.85; N, 16.20; S, 12.44%.

2-((4-Cyano-1-morpholin-4-yl-6,7-dihydro-5*H*-cyclopenta[*c*]pyridin-3- yl) sulfanyl) acetamide (III). A mixture of cyclopenta[*c*]pyridine thione (**II**) (3.00 g, 0.015 mol) and fused sodium acetate (3.00 g, 0.034 mol) and chloroacetamide (1.40 g, 0.015 mol) in ethanol (40 mL) was refluxed for 1 h. The reaction mixture was allowed to cool. The solid precipitate was collected and recrystallized from ethanol as yellow crystals in 69% (2.50 g) yield, mp 208–210°C. FT-IR: 3393, 3185 (NH_2), 2969, 2920, 2844 (CH aliphatic), 2203 (CN), and 1689 (CO amide). ^1H NMR (300 MHz, DMSO-d_6): 2.01 (m, 2H, CH_2: C6 cyclopenteno), 2.82 (m, 2H, CH_2: C7 cyclopenteno), 2.89 (m, 2H, CH_2: C5 cyclopenteno), 3.32 (m, 4H, $2CH_2$: $(CH_2)_2N$ morpholinyl), 3.32 (m, 4H, $2CH_2$: $(CH_2)_2O$ morpholinyl), 3.81 (s, 2H, CH_{2amide}), and 7.10–7.48 (s, 2H, $CONH_2$). Anal. calcd. for: $C_{15}H_{18}N_4O_2S$ (318.40) C, 56.59; H, 5.70; N, 17.60; S, 10.07%. Found: C, 56.68; H, 5.58; N, 17.48; S, 9.95%.

1-Amino-5-morpholin-4-yl-7,8-dihydro-6*H*-cyclopenta[*d*]thieno[2,3-*b*]pyridine-2-carboxamide (IV). Method A. To a stirred solution of the sulfanyl acetamide (**III**) (2.75g, 0.01 mol) in absolute ethanol (10 mL), a few drops of sodium ethoxide solution (prepared from 0.50 g of finely cut sodium metal dissolved in absolute ethanol) was added. The mixture was refluxed with stirring for 10 min. The precipitated solid that separated out during reflux was collected, dried, and recrystallized from ethanol as white crystals in 80% (2.20 g) yield, mp 248–250°C.

Method B. A mixture of the cyclopenta[*c*]pyridine thione (**II**) (2.75 g, 0.01 mol) and chloroacetamide (0.94 g, 0.01 mol) in ethanol (40 mL) and anhydrous

potassium carbonate (3.00 g, 22.0 mmol) was stirred with reflux for 3 h, then the mixture was allowed to cool. The solid product formed was filtered off, washed with water, dried, and recrystallized from ethanol as white crystals in 55% (1.50 g) yield, mp 248–250°C. FT-IR: 3447, 3315, 3136 ($2NH_2$), 2961, 2863 (CH aliphatic), and 1647 (CO amide). ^{1}H NMR (300 MHz, DMSO-d_6): 2.07 (m, 2H, CH_2: C7 cyclopenteno), 2.83 (m, 2H, CH_2: C6 cyclopenteno), 3.22 (m, 2H, CH_2: C8 cyclopenteno), 3.34 (m, 4H, $2CH_2$: $(CH_2)_2$N morpholinyl), 3.70 (m, 4H, $2CH_2$: $(CH_2)_2$O morpholinyl), 6.62 (s, 2H, $NH_{2thieno}$), 6.92 (s, 2H, $CO\underline{NH}_{2amide}$). ^{13}C NMR (75 MHz, DMSO-d_6): 23.74 (C7), 36.35 (C8), 36.59 (C6), 53.01 (C2', C6' morpholinyl), 71.35 (C3', C5' morpholinyl), 100.18 (C8b), 123.13 (C6a), 129.19 (C2), 152.4 (C8a), 156.21 (C1), 161.26 (C3a), 162.82 (C5), 172.59 (C9: CO amide). EI-MS (*m/z*): 318.18 [M^+]. Anal. calcd. for: $C_{15}H_{18}N_4O_2S$ (318.40): C, 56.59; H, 5.70; N, 17.60; S, 10.07%. Found: C, 56.70; H, 5.82; N, 17.36; S, 10.20%.

Ethyl 2-(4-(morpholin-4-yl)-7-oxo-2,3,7,8-tetrahydro-1*H*-cyclopenta[4',5']pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidin-9-yl)acetate (V). A suspension of the aminocarboxamide compound (**IV**) (0.50 g, 1.57 mmol) and diethyl malonate (2.00 mL, 12.50 mmol) was refluxed under solvent-free conditions for 15 min. Then ethanol (10 mL) was added. The reaction mixture was refluxed for additional 1 h. The solid product that separated out during reflux was filtered off and recrystallized from dioxane as pale yellow crystals in 69% (0.45 g) yield, mp >360°C. FT-IR: 3448 (NH), 2920 (CH aliphatic), 1741 (CO ester), 1655 (CO amide), and 1577 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6): 1.20 (t, 3H, J = 7.10 Hz, CH_3 ester), 2.03 (m, 2H, CH_2: C2 cyclopenteno), 2.85 (m, 2H, CH_2: C3 cyclopenteno), 3.20 (m, 2H, CH_2: C1 cyclopenteno), 3.45 (m, 4H, $2CH_2$: $(CH_2)_2$N morpholinyl), 3.69 (m, 4H, $2CH_2$: $(CH_2)_2$O morpholinyl), 3.77 (s, 2H, $\underline{CH_2}CO$), 4.13 (q, J = 7.10 Hz, 2H, CH_2 ester), and 12.69 (s, 1H, NH pyrimidinone). ^{13}C NMR (75 MHz, $CDCl_3$): 14.48 (C15: CH_3 ester), 25.53 (C2), 31.82 (C1), 32.48 (C3), 47.27 (C11: $\underline{CH_2}CO$), 47.93 (C2', C6'), 61.42 (C14), 66.59 (C3',C5'), 116.82 (C3a), 118.59 (C10a), 124.91 (C6a), 152.46 (C10a), 153.13 (C10c), 158.09(C9), 158.32(C5a), 159.83(C7), 166.04(C4), and 168.52(C12: CO ester). Anal. calcd. for: $C_{20}H_{22}N_4O_4S$ (414.48): C, 57.96; H, 5.35; N, 13.52; S, 7.73%. Found: C, 58.05; H, 5.40; N, 13.64; S, 7.82%.

2-(4-Morpholin-4-yl-7-oxo-2,3,7,8-tetrahydro-1*H*-cyclopenta[4',5']pyrido[3',2':4,5]thieno [3,2-*d*]pyrimidin-9-yl)acetohydrazide (VI). A solution of the ester compound (**V**) (0.50 g, 1.20 mmol) and hydrazine hydrate (1.00 mL, 0.02 mol) was refluxed in absence of solvent under neat conditions for 20 min, followed by addition of ethanol (20 mL). Then reflux was continued for additional for 2 h. The solid product that formed during reflux was filtered off, dried, and recrystallized from ethanol to give white crystals in 93% (0.40 g) yield, mp 238–240°C. FT-IR: 3447, 3314, 3135 (NH, NH_2), 2960, 2921, 2863 (CH aliphatic), 1648 (CO), and 1574 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6): 2.1 (m, 2H, CH_2: C2 cyclopenteno), 2.88 (m, 2H, CH_2, C3 cyclopenteno), 3.39 (m, 2H, CH_2: C1 cyclopenteno), 3.39 (s, 2H, $\underline{CH_2}CO$), 3.51 (m, 4H, $2CH_2$: $(CH_2)_2$N morpholinyl), 3.73 (m, 4H, $2CH_2$: $(CH_2)_2$O morpholinyl), 6.60 (s, 2H, NH_2), 6.84 (s, 1H, NH), 12.21 (s, 1H, NH pyrimidinone). ^{13}C NMR (100 MHz, DMSO-d_6): 25.41 (C2), 31.62 (C1), 31.87 (C3), 48.0 (C11), 48.3 (C2', C6'), 66.62 (C3', C5'), 95.6 (C3a), 118.43 (C10b), 124.39 (C6a), 147.62 (C10a), 151.49 (C10c), 156.58 (C9), 158.09 (C7: CO pyrimidine), and 167.81 (C12: CONH). Anal. calcd. for: $C_{18}H_{20}N_6O_3S$ (400.46): C, 54.23; H, 3.98; N, 23.71; S, 9.05%. Found: C, 54.20; H, 3.85; N, 23.80; S, 9.07%.

4'-Morpholin-4-yl-2',3'-dihydro-1'*H*,8'*H*-spiro-[cycloalkane-1,9'-cyclopenta[4',5']pyrido[3',2':4,5]-thieno[3,2-*d*]pyrimidin]-7'(10'*H*)-one (VIIa,b). General procedure. A sample of amino carboxamide (**IV**) (0.50 g, 1.30 mmol) and cycloalkanone (1.50 mmol) in acetic acid (3 mL) was heated under reflux for 30 min. The solid product that separated out on hot during reflux was filtered and recrystallized from ethanol.

4'-Morpholin-4-yl-2',3'-dihydro-1'*H*,8'*H*-spiro-[cyclopentane-1,9'-cyclopenta[4',5']pyrido[3',2':4,5]-thieno[3,2-*d*]pyrimidin]-7'(10'*H*)-one (VIIa). Cyclopentanone (EtOH), pale brown crystals in 83% (0.50 g) yield, mp 300–302°C, IR: 3393, 3243 (2NH), 2958 (CH aliphatic), and 1632 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6): 1.69–1.72 (m, 4H, $2CH_2$: C3, C4 cyclopentane), 1.78–1.86 (m, 4H, $2CH_2$: C2, C5 cyclopentane), 2.05 (m, 2H, CH_2: C2` cyclopenteno), 2.88 (m, 2H, CH_2: C3' cyclopenteno), 3.21 (m, 2H, CH_2: C1' cyclopenteno), 3.39 (m, 4H, $2CH_2$: $(CH_2)_2$N morpholinyl), 3.71 (m, 4H, $2CH_2$: $(CH_2)_2$N morpholinyl), 6.26 (s, 1H, NH), and 7.79 (s, 1H, $CO\underline{NH}$). ^{13}C NMR (75 MHz, DMSO-d_6): 22.56 (C2, C3), 25.42 (C2'), 31.62 (C1'), 32.11 (C3'), 38.58 (C2, C5), 48.22 (C2", C6"), 66.59 (C3", C5"), 78.49 (C9'), 103.69 (C3'a), 117.36 (C10'c), 124.41 (C6'a), 145.15 (C10'), 151.86 (C10'c), 157.73 (C5'a), 159.39 (C4'), 162.46 (C7': CO pyrimidine). EI-MS (*m/z*): 384.22 [M^+]. Anal. calcd. for $C_{20}H_{24}N_4O_2S$ (384.5): C, 62.48; H, 6.29; N, 14.57; S, 8.34%. Found: C, 62.44; H, 6.15; N, 14.39; S, 8.37%.

4'-Morpholin-4-yl-2',3'-dihydro-1'*H*,8'*H*-spiro-[cyclohexane-1,9'-cyclopenta[4',5']pyrido [3',2':4,5]-thieno[3,2-*d*]pyrimidin]-7'(10'*H*)-one (VIIb). Cyclohexanone (EtOH), as pale orange crystals in 80% (0.50 g) yield, mp 360°C, IR: 3255 (NH), 3144 (NHCO), 2928, 2845 (CH aliphatic) and 1636 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6): 1.26 (m, 2H, CH_2: C4 cyclohexane), 1.57 (m, 4H, $2CH_2$: C2, C6 cyclohexane), 1.57 (m, 2H, CH_2: C2' cyclopenteno), 2.06 (m, 4H, $2CH_2$: C3, C5 cyclohexane), 2.89

(m, 2H, CH_2: C3' cyclopenteno), 3.26 (m, 2H, CH_2: C1' cyclopenteno), 3.38 (m, 4H, $2CH_2$: $(CH_2)_2N$ morpholinyl), 3.71 (m, 4H, $2CH_2$: $(CH_2)_2O$ morpholinyl), 5.99 (s, 1H, NH) and 7.61 (s, 1H, NH). Anal. calcd. for $C_{21}H_{26}N_4O_2S$ (398.53): C, 63.29; H, 6.58; N, 14.06; S, 8.04%. Found: C, 63.19; H, 6.42; N, 14.18; S, 8.15%.

4-Morpholin-4-yl-2,3-dihydro-1*H*-cyclopenta-[4',5']pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidin-7(8*H*)-one (VIII). To a solution of the amino carboxamide (**IV**) (2.00 g, 6.00 mmol) and triethyl orthoformate (4.00 mL, 0.027 mol), a catalytic amount of glacial acetic acid (1 mL) was added. The reaction mixture was heated under reflux for 30 min. The solid precipitate that formed on hot during reflux was filtered off, dried, and recrystallized from dioxane as white needles in 90% (1.80 g) yield, mp 360°C. FT-IR: 3445 (NH broad), 2962, 2839 (CH aliphatic), 1640 (CO amide) and 1580 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6): 2.11 (m, 2H, CH_2: C2 cyclopenteno), 2.95 (m, 2H, CH_2: C3 cyclopenteno), 3.31 (m, 2H, CH_2: C1 cyclopenteno), 3.50 (m, 4H, $2CH_2$: $(CH_2)_2N$ morpholinyl), 3.73 (m, 4H, $2CH_2$: $(CH_2)_2O$ morpholinyl), 8.24 (s, 1H, CH pyrimidinone), 12.60 (s, 1H, NH pyrimidinone). Anal. calcd. for $C_{16}H_{16}N_4O_2S$ (328.39) C, 58.52; H, 4.91; N, 17.06; S, 9.76%. Found: C, 58.40; H, 5.08; N, 17.20; S, 9.88%.

1-(2-Chloroacetylamino)-5-morpholin-4-yl-7,8-dihydro-6*H*-cyclopenta[*d*]thieno[2,3-*b*]pyridine-2-carboxamide (IX). The amino-carboxamide (**IV**) (2.00 g, 6.00 mmol) and chloroacetyl chloride (0.80 mL, 0.01 mol) in dioxane (15 mL) was heated on water bath at 60–70°C for 2 h. The solid precipitate that formed on cooling and pouring on diluted sodium carbonate solution (10%) was collected and recrystallized from ethanol as pale yellow crystals in 64% (1.60 g) yield, mp 220–220°C. FT-IR: 3450, 3320, 3149 (NH, NH_2), 2957, 2920, 2849 (CH aliphatic), and 1651 (CO amide). ^{1}H NMR (300 MHz, DMSO-d_6): 2.08 (m, 2H, CH_2: C7 cyclopenteno), 2.86 (m, 2H, CH_2: C6 cyclopenteno), 3.24 (m, 2H, CH_2: C8 cyclopenteno), 3.35 (m, 4H, $2CH_2$: $(CH_2)_2N$ morpholinyl), 3.72 (m, 4H, $2CH_2$: $(CH_2)_2O$ morpholinyl), 4.62 (s, 2H, CH_2 Cl) , 6.62 (s, 1H, NH), and 6.91 (s, 2H, NH_2 amide). Anal. calcd. for $C_{17}H_{19}ClN_4O_3S$ (394.87): C, 51.71; H, 4.85; Cl, 8.98; N, 14.19; S, 8.12. Found: C, 51.56; H, 5.00; Cl, 9.10; N, 14.00; S, 8.25%.

9-(Chloromethyl)-4-morpholin-4-yl-2,3-dihydro-1*H*-cyclopenta[4',5']pyrido[3',2':4,5]thieno[3,2-*d*]-pyrimidin-7(8*H*)-one (X). Method A. Chloroacetyl amino derivative (**IX**) (3.50 g, 0.01 mol) in acetic anhydride (20 mL) was refluxed for 2 h. The solid product that separated out during reflux was filtrated off, dried, and recrystallized from dioxane as pale yellow crystals in 51% (1.60 g), yield, mp >360°C.

Method B. A mixture of the amino carboxamide (**IV**) (3.50 g, 0.01 mol) and excess amount of chloroacetyl chloride (5.00 mL, 0.064 mol) was heated on water bath at 60–70°C for 5 h, then poured into cold water (100 mL), and neutralized with sodium carbonate solution (10%) to just alkaline. The solid precipitate formed was recrystallized from dioxane as pale yellow crystals in 61% (2.00 g) yield, mp >360°C. FT-IR: 3438 (NH), 2845 (CH aliphatic), and 1655 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6): 2.13 (m, 2H, CH_2: C2 cyclopenteno), 2.97 (m, 2H, CH_2: C3 cyclopenteno), 3.36 (m, 2H, CH_2: C1 cyclopenteno), 3.52 (m, 4H, $2CH_2$: $(CH_2)_2N$ morpholinyl), 3.74 (m, 4H, $2CH_2$: $(CH_2)_2O$ morpholinyl), 4.62 (s, 2H, CH_2Cl), 12.21 (s, 1H, NH). Anal. calcd. for $C_{17}H_{17}ClN_4O_2S$ (376.86): C, 54.18; H, 4.55; Cl, 9.41; N, 14.87; S, 8.51%. Found: C, 54.27; H, 4.69; Cl, 9.34; N, 14.73; S, 8.64%.

4-Morpholin-4-yl-9-(alkyl(aryl)amino)methyl-2,3-dihydro-1*H*-cyclopenta[4',5']pyrido[3',2':4,5]thieno-[3,2-*d*]pyrimidin-7(8*H*)-one (XIa–c). General procedure. A mixture of the chloromethyl pyrimidinone (**X**) (1.00 g, 2.65 mmol) and the corresponding amine (2.80 mmol) was refluxed under solvent-free conditions for 2 h. The solid product which separated out on hot during reflux was filtrated off, dried, and recrystallized from the appropriate solvent.

4-Morpholin-4-yl-9-((phenylamino)methyl)-2,3-dihydro-1*H*-cyclopenta[4',5']pyrido[3',2': 4,5]thieno-[3,2-*d*]pyrimidin-7(8*H*)-one (XIa). Obtained by the reaction with aniline as white crystals in 78% (0.90 g) yield, mp 290–292°C, IR: 3318 (2NH), 3020 (CH aromatic), 2957 (CH aliphatic), and 1643 (C=O amide). ^{1}H NMR (400 MHz, DMSO-d_6): 2.11 (m, 2H, CH_2: C2 cyclopenteno), 2.94 (m, 2H, CH_2: C3 cyclopenteno), 3.53 (m, 2H, CH_2: C1 cyclopenteno), 3.53 (m, 4H, $2CH_2$: $(CH_2)_2N$ morpholinyl), 3.73 (m, 4H, $2CH_2$: $(CH_2)_2O$ morpholinyl), 4.32 (s, 2H, $\underline{CH_2}NH$), 6.06 (s, 1H, $\underline{NH}Ph$), 6.56–7.1 (m, 5H, ArH), 12.55 (s, 1H, NH pyrimidinone). Anal. calcd. for $C_{23}H_{23}N_5O_2S$ (433.53): C, 63.72; H, 5.35; N, 16.15; S, 7.40 Found: C, 63.58; H, 5.50; N, 16.00; S, 7.53%.

4-Morpholin-4-yl-9-((morpholin-4-yl)methyl)-2,3-dihydro-1*H*-cyclopenta[4',5']pyrido[3',2':4,5]thieno-[3,2-*d*]pyrimidin-7(8*H*)-one (XIb). Obtained by the reaction with morpholine as yellow crystals in 70% (0.80 g) yield, mp 340–342°C, IR: 3438 (NH), 2957, 2919, 2849 (CH aliphatic) and 1648 (C=O amide). ^{1}H NMR (400 MHz, DMSO-d_6): 2.13 (m, 2H, CH_2: C2 cyclopenteno), 2.58 (m, 4H, $2CH_2$: C2", C6" morpholinyl), 2.97 (m, 2H, CH_2: C3 cyclopenteno), 3.28 (m, 2H, CH_2: C1 cyclopenteno), 3.36 (s, 2H, CH_2N morpholinyl), 3.56 (m, 4H, $2CH_2$: C2', C6' morpholinyl), 3.62 (m, 4H, $2CH_2$: C3", C5" morpholinyl), 3.74 (m, 4H, $2CH_2$: C3', C5' morpholinyl), 12.26 (s, 1H, NH). Anal. calcd. for $C_{21}H_{25}N_5O_3S$ (427.52): C, 59.00; H, 5.89; N, 16.38; S, 7.50 Found: C, 59.14; H, 6.00; N, 16.52; S, 7.64%.

4-Morpholin-4-yl-9-((piperidin-1-yl)methyl)-2,3-dihydro-1*H*-cyclopenta[4',5']pyrido[3',2':4,5]thieno-[3,2-*d*]pyrimidin-7(8*H*)-one (XIc). Obtained by the reaction with piperidine as yellow crystals in 79%

(0.90 g), mp 300–302°C. IR: 3438 (NH), 2935, 2850 (CH aliphatic), and 1651 (C=O amide). ^{1}H NMR (400 MHz, DMSO-d_6): 1.38 (m , 2H ,CH_2: C4" piperidinyl), 1.53 (m, 4H, $2CH_2$: C3", C5" piperidinyl), 2.01 (m, 4H, $2CH_2$: C2", C6" piperidinyl), 2.14 (m, 2H, CH_2: C2 cyclopenteno), 2.95 (m, 2H, CH_2: C3 cyclopenteno), 3.28 (m, 2H, CH_2: C1 cyclopenteno), 3.49 (s, 2H, CH_2N), 3.51 (m, 4H, $2CH_2$: $(CH_2)_2N$ morpholinyl), 3.74 (m, 4H, $2CH_2$: $(CH_2)_2O$ morpholinyl), 12.14 (s, 1H, NH). Anal. calcd. for $C_{22}H_{27}N_5O_2S$ (425.55): C, 62.09; H, 6.40; N, 16.46; S, 7.53. Found: C, 62.20; H, 6.28; N, 16.35; S, 7.43%.

4-Morpholin-4-yl-11-phenyl-2,3,11,12-tetrahydrocyclopenta[4',5']pyrido[3",2":4',5']thieno [3',1':4,5]-pyrimido[1,2-*a*]pyrazine-7,10(1*H*,9*H*)-dione (XII). The pyrimidinone (**XIa**) (0.50 g, 1.20 mmol) and chloroacetyl chloride (1.00 mL, 1.30 mmol) were heated at 60–70°C under solvent-free conditions for 2 h. The solid product that was obtained by pouring on diluted sodium carbonate solution (10%) was filtrated off, dried, and recrystallized from dioxane as white crystals in 73% (0.40 g) yield, mp 238–240°C. FT-IR: 2956, 2850 (CH aliphatic), 1684, 1647 (2CO), and 1582 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6): 2.12 (m, 2H, CH_2: C2 cyclopenteno), 2.97 (m, 2H, CH_2: C3 cyclopenteno), 3.27 (m, 2H, CH_2: C1 cyclopenteno), 3.51 (m, 4H, $2CH_2$: N morpholinyl), 3.74 (m, 4H, $2CH_2$: O morpholinyl), 4.15 (s, 2H, C-$\underline{CH_2}$-N pyrazine), 4.84 (s, 2H, N-$\underline{CH_2}$-CO pyrazine), and 7.42–7.66 (m, 5H, ArH). Anal. Calcd. for $C_{25}H_{23}N_5O_3S$ (473.15): C, 63.41; H, 4.90; N, 14.79; S, 6.77. Found: C, 64.26; H, 5.05; N, 14.88; S, 6.90%.

4-Morpholin-4-yl-10-phenyl-2,3,10,11-tetrahydro-1*H*-cyclopenta[4',5']pyrido[3',2':4,5]thieno[3,2-*d*]-imidazo[1,5-*a*]pyrimidin-7(9*H*)-one (XIII). The phenylaminomethyl pyrimidinone (**XIa**) (0.50 g, 1.20 mmol) was dissolved in warm dioxane (10 mL) and then formaldehyde (1 mL, 0.033 mol, 35%) was added dropwise with stirring for 5 min. The reaction mixture was heated at 50–60°C with stirring for 2 h. The solid precipitate that separated out during reflux was recrystallized from dioxane to afford white crystals in 60% (0.30 g) yield, mp 360°C. FT-IR: 2942, 2837 (CH aliphatic), 1675 (CO) and 1599 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6): 2.12 (m, 2H, CH_2: C2 cyclopenteno), 2.97 (m, 2H, CH_2: C3 cyclopenteno), 3.48 (m, 2H, CH_2: C1 cyclopenteno), 3.50 (m, 4H, $2CH_2$: $(CH_2)_2N$ morpholinyl), 3.74 (m, 4H, $2CH_2$: $(CH_2)_2O$ morpholinyl), 4.12 (s, 2H, C-$\underline{CH_2}$-N imidazole), 4.88 (s, 2H, N–$\underline{CH_2}$–N imidazole), and 7.29–7.42 (m, 5H, ArH). Anal. Calcd. for $C_{24}H_{23}N_5O_2S$ (445.54): C, 64.70; H, 5.20; N, 15.72; S, 7.20%. Found: C, 64.84; H, 5.35; N, 15.60; S, 7.08%.

4-Morpholin-4-yl-7-oxo-1,2,3,8-tetrahydro-1*H*-cyclopenta[4',5']pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidin-9(10*H*)-thione (XIV). A mixture of amino carboxamide (**IV**) (0.50 g, 1.60 mmol) and carbon disulphide (1.50 mL, 0.02 mol) in anhydrous pyridine (3 mL) was refluxed on a steam bath at 100°C for 5 h. The solid precipitate which formed during reflux was collected and recrystallized from ethanol as green needles in 76% (0.38 g) yield, mp 3°C. FT-IR: 3369, 3490 (2NH), 2950, 2840 (CH aliphatic), 1656 (C=O), 1581 (C=N), and 1239 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6): 2.09 (m, 2H, CH_2: C2 cyclopenteno), 2.94 (m, 2H, CH_2: C3 cyclopenteno), 3.29 (m, 2H, CH_2: C1 cyclopenteno), 3.35 (m, 4H, $2CH_2$: $(CH_2)_2N$ morpholinyl), 3.72 (m, 4H, $2CH_2$: $(CH_2)_2O$ morpholinyl), 11.72 (s, 1H, CO$\underline{NH}$), 12.60 (s, 1H, $\underline{NH}$CS). Anal. Calcd. for $C_{16}H_{16}N_4O_2S_2$ (360.45): C, 53.32; H, 4.47; N, 15.54; S, 17.79. Found: C, 53.45; H, 4.60; N, 15.68; S, 17.94%.

9-Alkylsulfanyl-4-morpholin-4-yl-7-oxo-2,3,7,8-tetrahydro-1*H*-cyclopenta[4',5']pyrido[3',2':4,5]thieno-[3,2-*d*]pyrimidine (XV a,b). General procedure. A mixture of the pyrimidine thione (**XIV**) (0.50 g, 1.30 mmol) and the alkylating agent (1.30 mmol) in ethanol (10 mL) and anhydrous potassium carbonate (0.06 g, 4.30 mmol) was refluxed for 2 h. The solid precipitate which formed on cooling was filtered, dried, and recrystallized from ethanol.

Ethyl 2-((4-morpholin-4-yl-7-oxo-2,3,7,8-tetrahydro-1*H*-cyclopenta[4',5']pyrido[3',2':4,5]thieno[3,2-d]-pyrimidin-9-yl)sulfanyl)acetate (XVa). Produced by the reaction with ethyl chloroacetate as yellow crystals in 67% (0.40 g) yield, mp 230–232°C. IR: 3454 (NH), 2959, 2917, 2849 (CH aliphatic), 1725 (C=O, unsat. ester), 1669 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6): 1.20 (t, 3H, J = 7.10 Hz, CH_3 ester), 2.12 (m, 2H, CH_2: C2 cyclopenteno), 2.99 (m, 2H, CH_2: C3 cyclopenteno), 3.38 (m, 2H, CH_2: C1 cyclopenteno), 3.52 (m, 4H, $2CH_2$: $(CH_2)_2N$ morpholinyl), 3.73 (m, 4H, $2CH_2$: $(CH_2)_2O$ morpholinyl), 4.14 (s, 2H, $\underline{CH_2}$CO), 4.17 (q, 2H, J = 7.10 Hz, CH_2 ester), 13.02 (s, 1H, NH pyrimidinone). Anal. calcd. for: $C_{20}H_{22}N_4O_4S_2$ (446.54): C, 53.80; H, 4.97; N, 12.55; S, 14.36. Found: C, 53.66; H, 5.10; N, 12.42; S, 14.50%.

4-Morpholin-4-yl-9-((2-oxo-2-phenylethyl)sulfanyl)-2,3-dihydro-1*H*-cyclopenta[4',5']pyrido [3',2':4,5]-thieno[3,2-d]pyrimidin-7(8*H*)-one (XVb). Produced by the reaction with phenacyl bromide as yellow crystals in 76% (0.50 g) yield, mp: 230–232°C. IR: 3315 (NH), 3447, 2946, 2917, 2834 (CH aliphatic), 1685(C=O, COPh), 1648 (C=O amide). ^{1}H NMR (400 MHz, DMSO-d_6): 2.10 (m, 2H, CH_2: C2 cyclopenteno), 2.86 (m, 2H, CH_2: C3 cyclopenteno), 3.37 (m, 2H, CH_2: C1 cyclopenteno), 3.57 (m, 4H, $2CH_2$: N morpholinyl), 3.73 (m, 4H, $2CH_2$: O morpholinyl), 4.70 (s, 2H, $\underline{CH_2}$CO), 6.16–7.99 (m, 5H, ArH), 8.11 (s, 1H, NH pyrimidinone). Anal. calcd. for: $C_{24}H_{22}N_4O_3S_2$ (478.59): C, 60.23; H, 4.63; N, 11.71; S, 13.40. Found: C, 60.34; H, 4.75; N, 11.60; S, 13.52%.

In Vitro Antibacterial Assay

All microorganisms used were obtained from the culture collection of Microbiology Department, Faculty of Medicine, Assiut University. Activities of several synthesized compounds against gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) bacterial strains were investigated using 5 mL of 100 μg/mL DMSO solutions of the tested compounds **(II)**, **(III)**, **(IV)**, **(V)**, **(IX)**, **(X)**, and **(XIa)** in DMSO as a solvent. The synthesized compounds were initially screened by a maximum concentration of 100 μg/mL in DMSO and an antibiotic drug (Ofloxacin) as a reference. The sterile medium (Nutrient Agar Medium, 15 mL) in each Petri dish was uniformly smeared with cultures of gram-positive and gram-negative bacteria. Antibacterial activity of the tested compounds was determined according to the disc diffusion method reported by Kwon-Chung and Bennett [31] using 5 mm diameter filter paper discs loaded with 50 μL of the solution under investigation. The minimum inhibitory concentration (MIC) of each compound was taken as the lowest concentration (μg/mL) that did not give any visible bacteria growth. The plates were incubated at 37 ± 2°C for 24 h and the zone of inhibition was determined.

In Vitro Antifungal Assay

The fungal strains (*Candida albicans*, *Aspergillus flavus*, *Geotrichum candidum*, and *Trichophyton rubrum*) were obtained from some cases of human dermatophytosis (Assiut University Mycological Center, AUMC). The fungal strains were grown in sterilized 9-cm Petri dishes containing Sabouraud's dextrose agar (SDA) supplemented with 0.05% of chloramphenicol to suppress bacterial contamination [32]. From these cultures, agar disks (10 mm diameter) containing spores were transferred aseptically to screw-topped vials containing 20 mL sterile distilled water. After shaking, 1 mL samples of the spore suspension were pipetted into sterile Petri dishes, followed by the addition of 15 mL liquefied SDA medium and then left to solidify. The tested compounds **(II)**–**(III)**, **(IV)**, **(V)**, **(IX)**, **(X)**, and **(XIa)** and the reference compound (clotrimazole) were dissolved in DMSO to give 100 μg/mL concentration. Antifungal activity was determined according to the disc diffusion method as reported by Kwon-Chung and Bennett [31] using 5 mm-diameter filter paper discs loaded with 50 μL of the solution under investigation (2.0%) and the inoculated plates were incubated at room temperature for 4 days. MIC of each compound was taken as the lowest concentration (mμg/mL) that did not give any visible fungi growth. The zone of inhibition was determined (Table 2).

CONCLUSION

In this article, we have provided an easy access for synthesis of novel cyclopenta[*d*]thieno[2,3-*b*]pyridine ring system fused to other heterocycles, namely, pyrimidine, imidazopyrimidine, and pyrimidopyrazine. From the antimicrobial results, we found that most of the examined novel cyclopentathienopyridines and thienocyclopentapyridopyrimidines exhibited promising antibacterial and antifungal activities and thus can be considered as potential antibacterial and antifungal drugs.

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving human participants performed by any of the authors and does not contain any studies involving animals performed by any of the authors.

SUPPLEMENTARY MATERIALS

Supplementary materials are available for this article at https://doi.org/10.1134/S1068162020010148 and are accessible for authorized users.

Conflict of Interests

The authors declare that they have no conflicts of interest.

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