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Original article

2-Aminothiophenes as building blocks in heterocyclic synthesis: Synthesis and antimicrobial evaluation of a new class of pyrido[1,2-*a*]thieno[3,2-*e*]pyrimidine, quinoline and pyridin-2-one derivatives

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ABSTRACT

Multisubstituted 2-aminothiophenes **1a**–**c** can be readily cyanoacylated *via* reaction with cyanoacetic acid in presence of acetic anhydride under a microwave irradiation to form the corresponding cyanoacetamides **2a**–**c**, which condensed with DMF-DMA to form the corresponding enamines **4** that reacted with hydrazine hydrate to yield the aminopyrazoles **5**. Moreover the cyanoacetamides **2a**–**c** reacted with a variety of arylidenmalononitrile to afford a novel pyrido[1,2-*a*]thieno[3,2-*e*]pyrimidine derivatives **12a**–**o**. In addition the enamines **4a,b** reacted with malononitrile to afford the pyrido[1,2-*a*]thieno[3,2-*e*]pyrimidine derivatives **19a**,**b**. The cyanoacetamides **2a**,**b** reacted also with salicylaldehyde to afford the quinoline derivatives **24a**,**b**. Moreover the cyanoacetamides **2a**,**b** reacted with the enaminones **25a**–**c** to form the corresponding Pyridin-2-one derivatives **29a**–**c**. Reactions of **2a**,**c** with bezenediazonium chloride afford the arylhydrazones **30a**,**b** that reacted with chloroacetonitrile to form the acyclic product **31** which could not be further cyclized to the corresponding 4-aminopyrazole. The X-ray crystallographic analyses of seven products could be obtained thus establishing with certainty the proposed structures in this work. Most of the synthesized compounds in this investigation were tested and evaluated as antimicrobial agents.

1. Introduction

Multisubstituted 2-aminothiophenes and thiophene containing compounds have demonstrated a broad spectrum of uses, including dyes, agrochemical applications [1] and they are known to exhibit various biological and pharmaceutical activities such as potential antioxidant and anti-inflammatory agents [2,3], anti-HIV PR inhibitors [4], anti-breast cancer [5], anthelmintic activity against haemonchus contortus [6], anti-avian influenza virus (H5N1) [7], a multitargeted kinase inhibitor [8], AMPK activators [9], antitubercular agent [10]. Multisubstituted 2-aminothiophenes are privileged structures, which attracted considerable attention in the designing of biologically active molecules [11–15]; the simplest and most convergent preparation of this class of compounds is the condensation of ketones with an activated nitriles and elemental sulfur in the presence of a base, which was first described in 1960s by Gewald and co-workers [16]. Although a one-pot procedure is well-established, the two-step procedure in which an α,β-unsaturated nitrile is first prepared by Knoevenagel condensation of a ketone or aldehyde with an activated nitriles [1,17], followed by base-promoted reaction with sulfur, has generally been found to result in higher yields (Eq. (1)). In the course of our current biological chemistry research, the multisubstituted 2-aminothiophenes will be utilized as building blocks in the synthesis of a new class of heterocyclic compounds with the evaluation of their biological activities.

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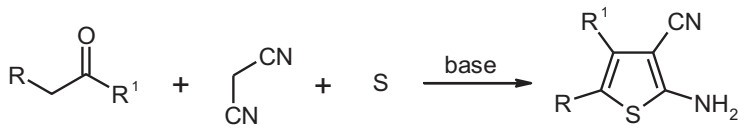


Eq. 1

1

2. Results and discussion

2.1. Synthetic chemistry

The amino group in the multisubstituted 2-aminothiophenes [16] **1** is feebly basic and can only be reacted with electrophiles by special technique and condition. Recently Slatt's [18] cyanoacetamide synthesis could be applied in our laboratories to synthesis a variety of a novel cyanoacetamides and heterocyclic compounds [19–22]. It appeared to us of value to investigate the reactivity of the amino group in the 2-aminothiophenes **1** which can be cyanoacylated to yield the corresponding cyanoacetamides *via* the same methodologies. The possibility of using these cyanoacetamides as precursor to a variety of novel thiophene derivatives and dyes will be investigated. The investigation aimed at this goal began with the reactions of **1** with a preheated mixture of acetic anhydride and cyanoacetic acid under microwave irradiation conditions to yield the corresponding cyanoacetamides **2**. The structure of **2a** was established based on X-ray crystallographic analysis (cf. Table 1 and Fig. 1). The cyanoacetamides **2a,b** react with dimethylformamide dimethylacetal (DMF-DMA) to yield the corresponding enamines **4a,b**. Although these processes have the potential of producing mixtures of the enamines stereoisomers **3** and **4**. The fact that only the *E*-isomer **4** could be formed was confirmed by using X-ray single crystal (cf. Fig. 2). The enamines **4a,b** react with hydrazine hydrate in refluxing dioxane to yield the corresponding aminopyrazoles **5a,b** (cf. Scheme 1).

The active methylene in the cyanoacetamides **2** underwent nucleophilic addition reaction to the double bond of a variety of arylidenmalononitrile **6** *via* a Michael type addition reaction, by refluxing in ethanol containing few drops of piperidine where a substance its structure should be either **7** or one of the two isomeric forms **12** or **14** was produced. The actual structure of the product was assigned as pyrido[1,2-*a*]thieno[3,2-*e*]pyrimidine derivatives **12** based on its ^{1}H NMR spectroscopic data which showed two NH signals and devoid of an amino group signal which should be appeared if the reaction product was **7** or **14**. Moreover **7** was excluded as reaction product since the ^{1}H NMR lacked the pyran H-4 signal which should appear at approximately $\delta = 4$–5 ppm. In addition the X-ray crystallographic analysis of this product demonstrated that it has the structure represented by **12** which is believed to be formed, most likely, *via* the intermediacy of **8**–**11** through the Dimroth rearrangement and aromatization of the latter to **12** (cf. Scheme 2, Figs. 3 and 4).

Table 1
Selected bond lengths and bond angles for **2a**.

Bond	Bond length(Å)	Bond	Bond angle(°)
C1–C2	1.359	C1–S1–C10	90.77
C2–C9	1.438	S1–C1–C2	114.44
C9–C10	1.379	C1–C2–C9	110.00
S1–C10	1.716	C2–C9–C10	113.04
S1–C1	1.715	S1–C10–C9	111.74
C10–N2	1.394	N2–C10–C9	124.78

In a similar manner, another class of pyrido[1,2-*a*]thieno[3,2-*e*] pyrimidine derivatives **19a**,**b** could be prepared by the reaction of enaminones **4a**,**b** with malononitrile in refluxing DMF containing few drops of piperidine *via* the intermediacy of **15**–**18** (cf. Scheme 3).

In contrast to the above observed chemistry the cyanoacetamides **2a**,**b** reacted with 2-(4-dimethyl- aminobenzylidene)malononitrile **20** to afford the arylidene derivatives **21**. Which could be also obtained *via* condensation of **2** with 4-dimethylaminobenzaldehyde in EtOH/piperidine to afford the arylidene derivatives **21**. (cf. Scheme 4, Fig. 5)

Moreover condensation of cyanoacetamides 2a,b with salicylaldehyde afforded the quinoline derivatives 24a,b through the intermediacy of 22 and 23. The structure of the products was confirmed by X-ray single crystal determination of 24a (cf. Scheme 5, Fig. 6) (Table 2).

The addition of a variety of enaminones **25a**–**c** to cyanoacetamide **2a** gave intermediate adduct **26**, which readily eliminates dimethylamine group to give **27** that cyclized to **28** before rearrangement to the final pyridine derivatives **29a**–**c**. The pyridone structure was confirmed *via* the ^{1}H-NMR spectra of the product which showed two NH signals one for the amide NH and the another one for the pyridone NH. Also the IR and ^{13}C-NMR spectra illustrate the presence of two CO and only one CN group (cf. Scheme 6).

The cyanoacetamides **2a**,**c** also coupled with the bezenediazonium chloride to yield the aryl hydrazones **30a**,**b**. It was found that the cyanoacetamides **2a** underwent coupling reaction with bezenediazonium chloride in pyridine while **2c** in EtOH/AcONa to form the corresponding arylhydrazones **30a**,**b**. The *E* conformation of the arylhydrazones was established *via* the X-ray structure determination of **30b**. In accordance with the literature reports [29,30] compound **30b** react with chloroacetonitriles in triethylamine to yield the acyclic product **31**. Trials to affect the cyclization of **31** into the corresponding 4-aminopyrazole **32** under variety of conditions was failed (cf. Scheme 7, Fig. 7) (Table 3).

3. Pharmacology

3.1. Methodology

The antimicrobial activities of 32 different chemical compounds were tested using Agar-well diffusion technique (Isaacson and

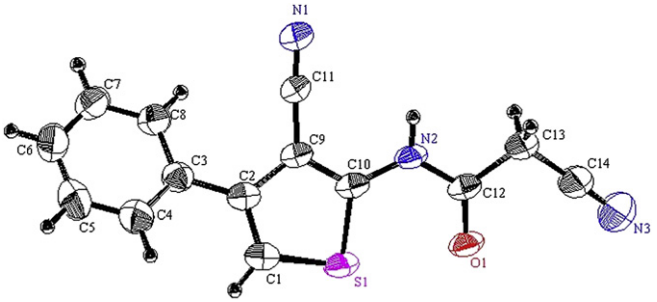


Fig. 1. ORTEP plot of the X-ray crystallographic data determined for **2a**. Crystallographic data have been deposited with the Cambridge crystallographic data Center as supplementary publication number CCDC 841496 [23].

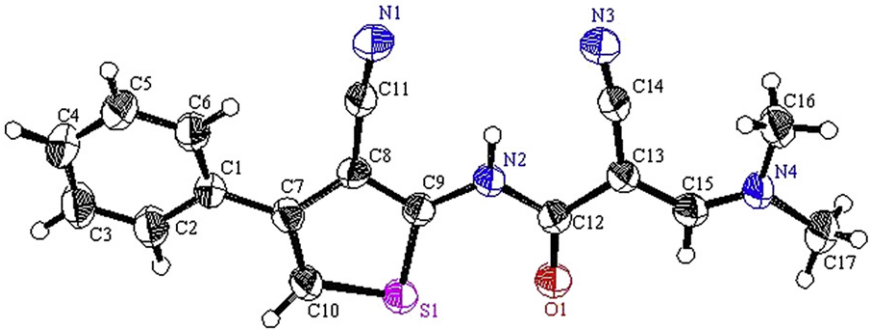
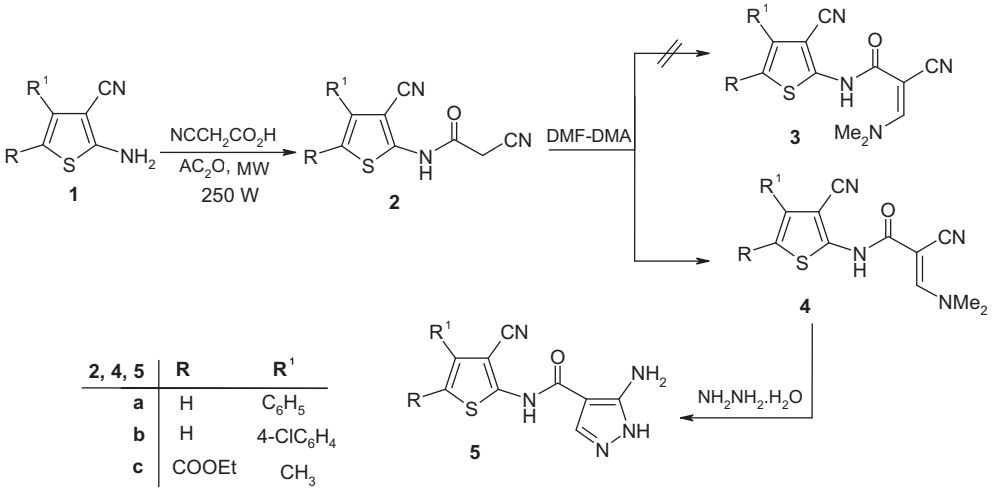


Fig. 2. ORTEP plot of the X-ray crystallographic data determined for **4a**. Crystallo- graphic data have been deposited with the Cambridge crystallographic data Center as supplementary publication number CCDC 841636 [24].

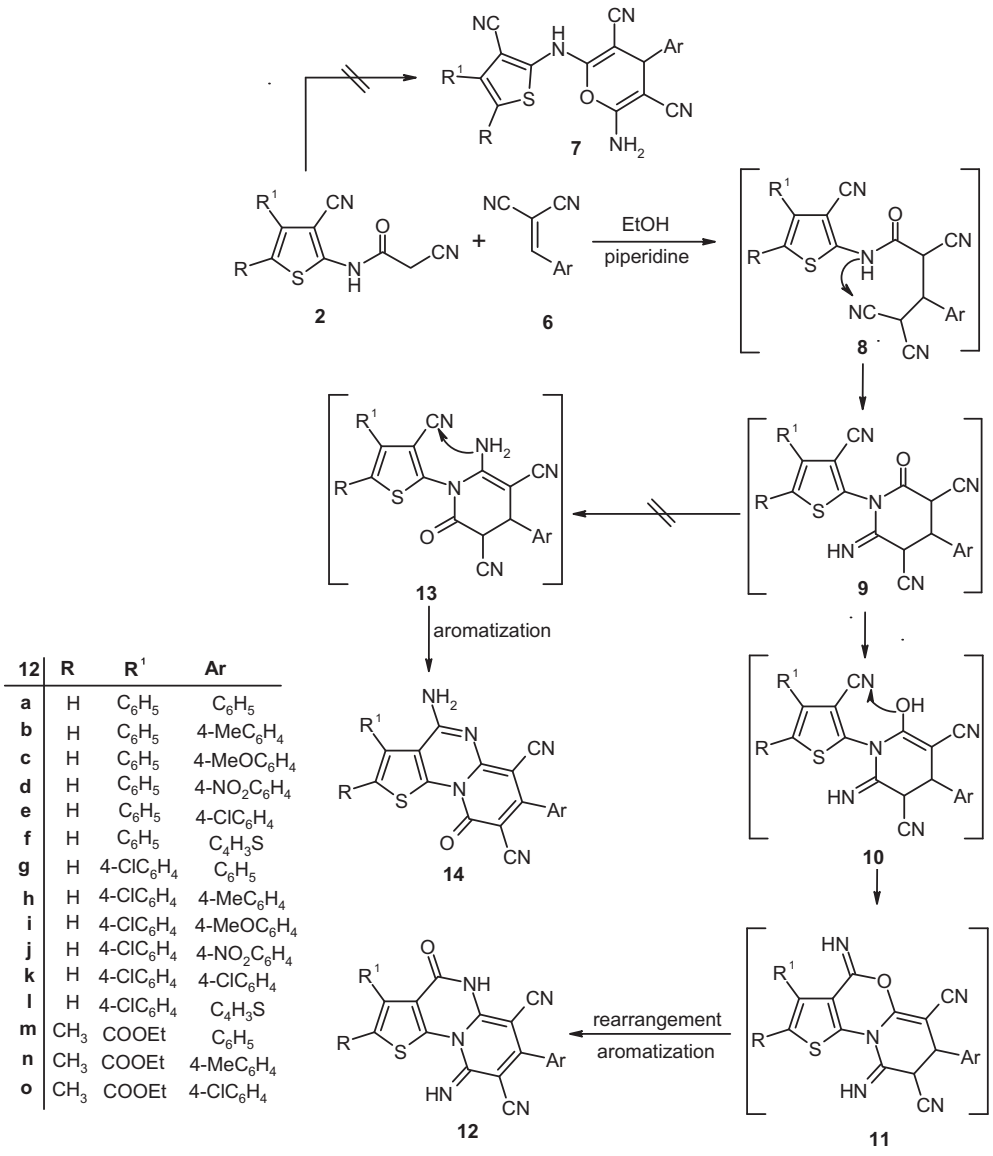
Kirchbaum, 1986) against 7 different microbial cultures obtained from Carolina Biological Supply (USA). Pure cultures of *Escherichia coli* (#124300), *Pseudomonas aeruginosa* (#155250A) and *Serratia marcescens* (#155449) (Gram negative bacteria), *Bacillus subtilis* (#154921) and *Staphylococcus aureus* (#155554A) (Gram positive bacteria) and *Candida albicans* (#155965) and *Saccharomyces cerevisiae* (#156250) (Yeast) were involved in the test. An aliquot of 0.1 ml of each bacterial strain was inoculated and spread on nutrient agar while 0.1 ml of the yeast was spread on potato dextrose agar (PDA). Agar-Well diffusion test was applied where 4 mm wells were produced by sterile cork borer and each well was inoculated with 100 μl of each of the tested chemicals with a final concentration of 1 mg ml^{-1}. Nutrient agar plates were incubated at 37 °C for 24 h while PDA plates were incubated at 25 °C for 48 h. The inhibition zones around the wells were measured and the average based on 3 replicas was recorded. For reference drugs 100 mg ml^{-1} of penicillin (Sigma) and cycloheximide (Sigma) were used as antibacterial and antifungal drugs respectively. The methodology shows the rational of using the two reference compounds i.e. the penicillin and the cycloheximide. The former is an active antibacterial agent because it works on the peptidoglycan that found only in the cell wall of bacteria and inhibit the bacterial cell wall synthesis by preventing the cross linking of peptides between the N-acetyl muramic acid and N-acetyl glucose amine [32]. On the other hand, the cycloheximide is an antifungal drug which works only on the eukaryotic cells and affects the RNA synthesis inhibiting the protein synthesis in yeasts [33] which subsequently leads to the inhibition of glycoprotein wall formation in these organisms [34]. Therefore, both penicillin and cycloheximide were utilized in the current study as reference compounds to compare the strength and the significance of the antimicrobial activities of the synthesized chemicals in comparison to established and utilized antimicrobial compounds .

3.2. Antimicrobial evaluation

The tested chemical compounds in this study showed variation in their antimicrobial activities, the results depicted in Table 4, show strong activities against Gram positive and Gram negative bacteria as well as against yeast. In addition, most of the chemical compounds in Table 5 showed moderate to week inhibition ability against most of the tested microorganisms as revealed by the diameters of their inhibition zones. The tested chemicals **1a**, **24a** and **24b** displayed strong inhibitory effects on the growth of *E. coli* and *B. subtilis* which showed inhibition zones exceeding 10 mm compared to the reference chemotherapeutic penicillin. Also compounds **1a**, **4b**, **19a** and **21a** exhibit strong activities against *S. marcescens* in comparison with the reference drug. Moreover compounds **1b**, **2c**, **5b** and **30b** revealed interesting high activities against the Gram positive bacteria *B. subtilis* with respect to the used reference drug. It's worth noticing that compounds **1a**, **12l** and **24a** strongly inhibited the growth of *C. albicans* when the cycloheximide failed to do. This microbe is known for its ability to cause diseases, either because it acts as primary pathogen or as an



Scheme 1. Synthesis of 5-amiopyrazole **5**.



12	R	R^1	Ar
a	H	C_6H_5	C_6H_5
b	H	C_6H_5	4-MeC_6H_4
c	H	C_6H_5	4-$MeOC_6H_4$
d	H	C_6H_5	4-$NO_2C_6H_4$
e	H	C_6H_5	4-ClC_6H_4
f	H	C_6H_5	C_4H_3S
g	H	4-ClC_6H_4	C_6H_5
h	H	4-ClC_6H_4	4-MeC_6H_4
i	H	4-ClC_6H_4	4-$MeOC_6H_4$
j	H	4-ClC_6H_4	4-$NO_2C_6H_4$
k	H	4-ClC_6H_4	4-ClC_6H_4
l	H	4-ClC_6H_4	C_4H_3S
m	CH_3	COOEt	C_6H_5
n	CH_3	COOEt	4-MeC_6H_4
o	CH_3	COOEt	4-ClC_6H_4

Scheme 2. Synthesis of pyrido[1,2-*a*]thieno[3,2-*e*]pyrimidine derivatives **12**.

opportunistic pathogen in humans with weak immune system. Inhibition zones for the Gram positive bacteria *S. aureus* were stronger than those recorded for *B. subtilis* in case of **1a**, **24a** and **30b**. Regarding the structure–activity relationship of the thiophenes against the microorganisms it was found that transformation of the aminothiophenes **1** into the corresponding cyanoacetamides **2** generally decrease the inhibitory effects while transformation of the latter into the corresponding enamines **4** caused slight increase in the biological effect like **4a** and not **4b** which have moderate growth inhibitory activities against

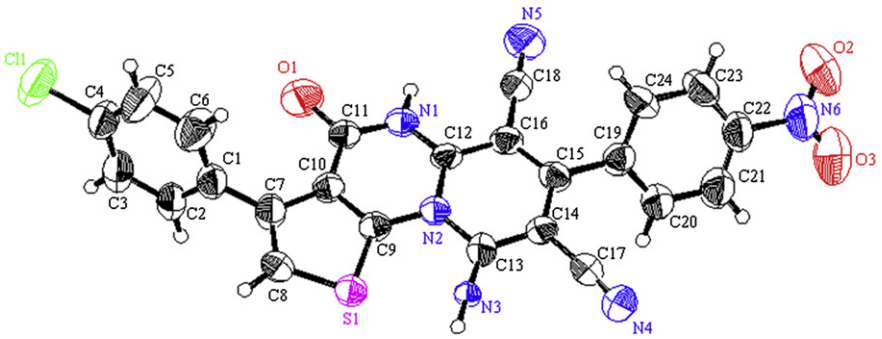


Fig. 3. ORTEP plot of the X-ray crystallographic data determined for **12j**. Crystallo- graphic data have been deposited with the Cambridge crystallographic data Center as supplementary publication number CCDC 841890[25].

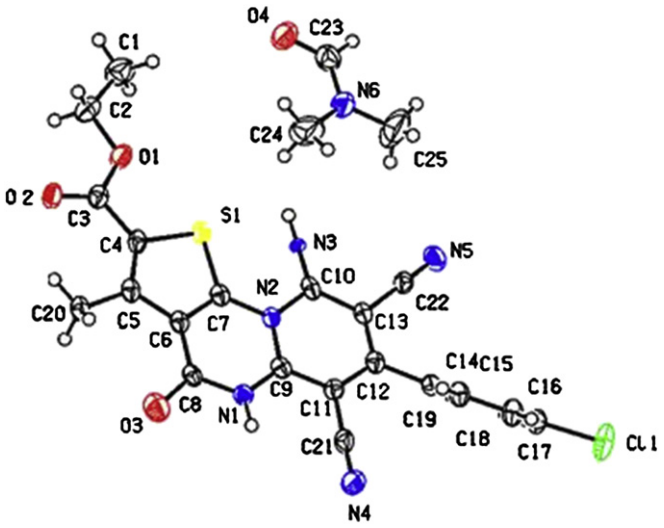


Fig. 4. ORTEP plot of the X-ray crystallographic data determined for **12o** containing a DMF molecule. Crystallographic data have been deposited with the Cambridge crystallographic data Center as supplementary publication number CCDC 847262 [26].

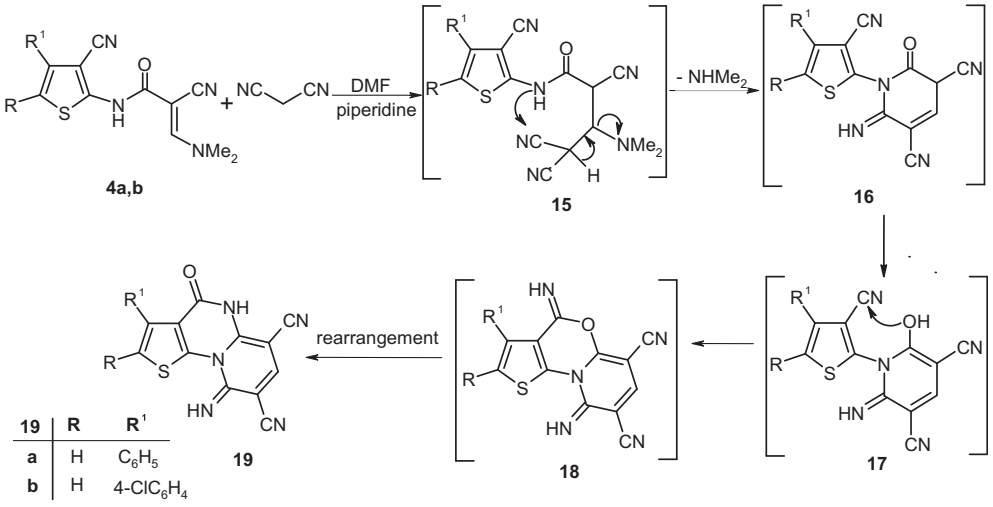
P. aeruginosa (Gram negative bacteria) as revealed by the diameters of their inhibition zones. In contrast, attachment of a pyrazole to the thiophene nucleus at position 2 *via* a carboxamide linker, exemplified by compounds **5a**,**b** unfortunately leads to a moderate antimicrobial effect. On other hand fusing the thiophene ring into the tricyclic system pyrido[1,2-*a*]thieno[3,2-*e*]pyrimidine derivatives **12a**–**o** and **19a**,**b** slightly enhance the antimicrobial activity since the majority of these compounds having moderate growth inhibitory activities against the tested organisms except compounds **12c**,**f**,**i** and **19a**,**b** which displayed a broad spectrum antibacterial profile only against the Gram negative bacteria *Pseudomonas aeruginosa,* while **12l** showed strong activity on both the Gram negative bacteria *P. aeruginosa* and the *C. albicans* (Yeast). It worth mentioning that the substances **24a**,**b**, possessing a quinoline ring appended to the C2 position of the thiophene nucleus *via* a carboxamide linker showed a high antimicrobial activity against the Gram negative bacteria *(Pseudomonas aeruginosa),* Gram positive bacteria (*B. subtilis* and *S. aureus)* and especially against Yeast (*C. albicans* and *S. cerevisiae*). In contrast linking a pyridine ring to the thiophene nucleus *via* a carboxamide linker in **29a**,**b** leads to moderate antimicrobial activity. Finally the conversion of the cyanoacetamides **2c** to the corresponding arylhydrazone **30b** displayed a broad spectrum antibacterial profile only against *B. subtilis* and *S. aureus* (Gram positive bacteria).

The results obtained for the synthesized compounds in this study support the findings of the other researchers who showed that chemicals with aminothiophenes building blocks and pyridothieno-pyrimidine core have various biological activities. Aminothiophenes were proved by many researchers for possessing biological effects including antimicrobial (bacterial, fungal, protozaol and viral) activities as well as for having antitumor and anti-inflammatory activities [35,36]. Where it has been reported that thiophenes derivatives act as allosteric enhancers of A1-adenosine receptor [37]. On the other hand, the synthesized chemical compounds with pyrido-thieno-pyrimidine core may act against cancer cells by acting as inhibitors for Cdc7 kinase which play critical roles in regulation of DNA replication in eukaryote cells [38]. Therefore, the new synthesized classes showed promising results for possessing the potentials to be utilized for medicinal purposes. Finally it is worth mention that despite the Cycloheximide, is strong antifungal compound the Candida albicans showed strong resistance to it in our study, which is compatible with the findings by many researchers [39–42].

4. Experimental

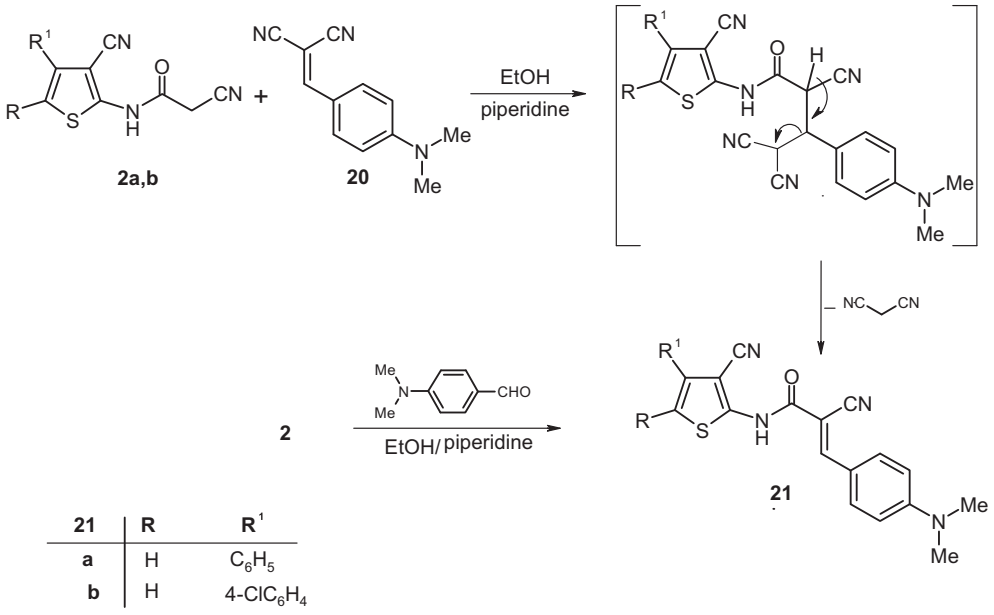
4.1. General

Melting points were recorded on a Griffin melting point apparatus and are reported uncorrected. IR spectra were recorded using KBr disks using a Perkin–Elmer System 2000 FT–IR spectrophotometer. ^{1}H NMR (400 MHz) or (600 MHz) and ^{13}C NMR (100 MHz) or (150 MHz) spectra were recorded at 25 °C or as reported in $CDCl_3$ or DMSO-d_6 as solvent with TMS as internal standard on a Bruker DPX 400 or 600 super-conducting NMR spectrometer. Chemical shifts are reported in ppm. Mass spectra were measured using a high resolution GC–MS (DFS) thermo spectrometers with EI (70 EV). Microanalyses were performed on a LECO CHNS-932 Elemental Analyzer. Follow up of the reactions and checking homogeneity of the prepared compounds was made by thin layer chromatography (TLC). Reactions were conducted under microwave irradiation in heavy-walled Pyrex tubes (capacity 10 mL) fitted with PCS caps. Microwave heating was carried out with



19	R	R^1
a	H	C_6H_5
b	H	4-ClC_6H_4

Scheme 3. Reaction of enamines **4a**,**b** with malononitrile.



21	R	R^1
a	H	C_6H_5
b	H	4-ClC_6H_4

Scheme 4. Reaction of **2a**,**b** with 2-(4-dimethylaminobenzylidene)malononitrile.

a single mode cavity Explorer Microwave synthesizer (CEM Corporation, 3100 Smith Farm Road, Matthews, NC, USA). The crystal structures were determined by a Rigaku R-AXIS RAPID diffractometer and Bruker X8 Prospector at Kuwait University. Compounds **1a**–**c** were prepared using literature procedures [16]. Also the enaminones **25a**–**c** were prepared according to the literature procedure [43].

4.2. General procedure for the preparation of cyanoacetamides *2a*–*c*

4.2.1. Method A (mw)

A solution of cyanoacetic acid (0.425 g, 5 mmol) in Ac_2O (5 mL) was heated in a microwave oven at a power of 250 W and 85 °C for 10 s then compounds **1a-c** (5 mmol) were added and the reaction mixture was heated for further 20 s at 100 °C. The reaction mixture was allowed to cool to room temperature and the formed crystalline solid was separated by filtration and washed with ethanol in case of **2a**,**b** and with water in case of **2c**.

4.2.2. Method B (thermally)

A solution of cyanoacetic acid (0.425 g, 5 mmol) in Ac_2O (10 mL) was heated at 100 °C for 5 min then compounds **1a**–**c** (5 mmol) were added and the reaction mixture was heated for further 30 min at 100 °C. The reaction mixture was allowed to cool to room temperature and the formed crystalline solid was separated by filtration and washed with ethanol in case of **2a**,**b** and with water in case of **2c.**

4.2.3. 2-Cyano-N-(3-cyano-4-phenylthiophen-2-yl)acetamide (*2a*)

Recrystallized from EtOH as creamy white crystals, yield: thermally (72%), by microwave (94%), m.p.: 233–234 °C; IR (KBr): νcm^{-1} 3225 (NH), 2215 (br, 2CN), 1668 (CO); ^{1}H NMR (DMSO-d_6): $\delta = 4.19$ (s, 2H, CH_2), 7.33 (s, 1H, thiophene H-5), 7.43 (t, $J = 7.6$ Hz, 1H, Ar–H), 7.49 (t, $J = 7.6$ Hz, 2H, Ar–H), 7.60 (d, $J = 7.6$ Hz, 2H, Ar–H) and 12.12 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6): $\delta = 26.13$ (CH_2), 92.99 (thiophene C-3), 114.57 (CN), 115.32 (CN), 115.94, 127.54, 128.36, 128.86, 133.48, 138.02, 150.41 and 162.11 ppm (Ar–C and CO); MS (EI): *m*/*z* (%) 267 (M^+, 46.35), 268 (M^++1, 9.75). Anal. calcd. for $C_{14}H_9N_3OS$ (267.31): C, 62.91; H, 3.39; N, 15.72; S, 12.00. Found: C, 62.85; H, 3.36; N, 15.79; S, 11.97.

4.2.4. Crystallographic analysis for *2a*

The crystals were mounted on a glass fiber. All measurements were performed on a Rigaku R-AXIS RAPID diffractometer using filtered Mo-Kα radiation. The data were collected at a temperature

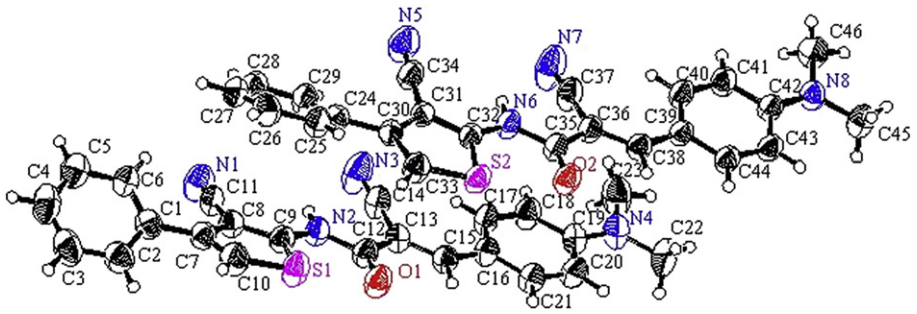
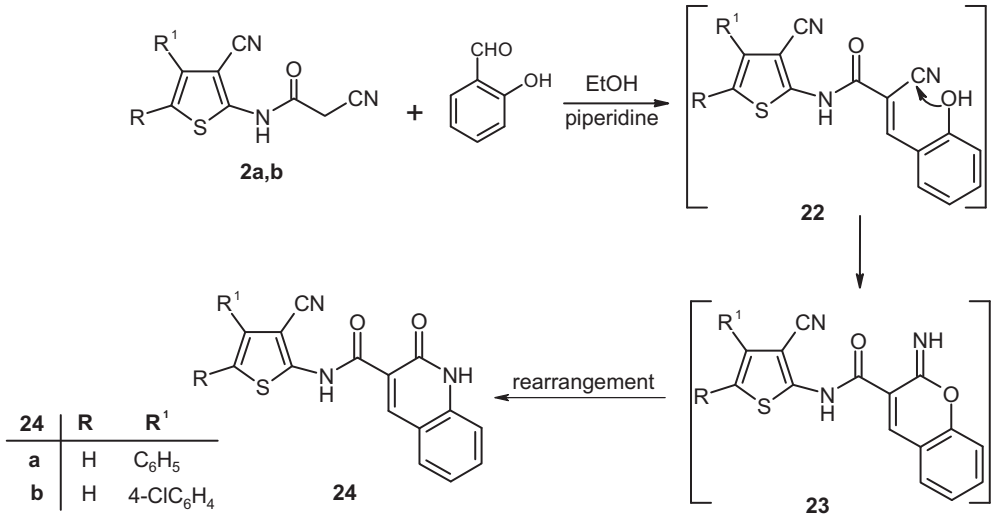


Fig. 5. ORTEP plot of the X-ray crystallographic data determined for **21a**. Crystallographic data have been deposited in the Cambridge crystallographic data Center as supplementary publication number CCDC 843132 [27].



Scheme 5. Reaction of cyanoacetamides **2a**,**b** with salicylaldehyde.

of 20 ± 1 °C to a maximum 2θ value of 55.0° using the ω scanning technique. The structure was solved by charge flipping method and expanded using Fourier techniques. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model.

4.2.5. Crystal data

$C_{14}H_9N_3OS$, M = 267.31, monoclinic, a = 9.4297(7) Å, b = 14.1478(6) Å, c = 9.4854(7) Å, V = 1261.7(2) Å^3, $\alpha = \gamma = 90.00°$, β = 94.402(7)°, space group: $P2_1/c$, Z = 4, D_{calc} = 1.407 g cm^{-3}, No. of reflection measured 2879, $2\theta_{max}$ = 54.9°, $R1$ = 0.036. Fig. 1 illustrates the structure as determined. Full data can be obtained on request from the CCDC [23].

*4.2.6. N-[4-(4-Chlorophenyl)-3-cyanothiophen-2-yl]-2-cyanoacetamide (**2b**)*

Recrystallized from an EtOH/dioxane (1:1) mixture as white crystals, yield: thermally (75%), by microwave (90%), m.p.: 226–228 °C; IR (KBr): νcm^{-1} 3231 (NH), 2218 (br, 2CN), 1697 (CO); ^{1}H NMR (DMSO-d_6): δ = 4.19 (s, 2H, CH_2), 7.39 (s, 1H, thiophene H-5), 7.56 (d, J = 8.0 Hz, 2H, Ar–H), 7.61 (d, J = 8.0 Hz, 2H, Ar–H) and 12.16 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 26.13 (CH_2), 92.67 (thiophene C-3), 114.41 (CN), 115.28 (CN), 116.49, 128.86, 129.30, 132.27, 133.14, 136.59, 150.49 and 162.16 ppm (Ar–C and CO); MS (EI): m/z (%) 301 (M$^+$, 45.17), 302 (M$^+$+1, 8.90). Anal. calcd. for $C_{14}H_8ClN_3OS$ (301.76): C, 55.73; H, 2.67; N, 13.93; S, 10.63. Found: C, 55.66; H, 2.74; N, 13.92; S, 10.65.

*4.2.7. Ethyl-4-cyano-5-(2-cyanoacetamido)-3-methylthiophene-2-carboxylate (**2c**)*

Recrystallized from EtOH as creamy white crystals, yield: thermally (68%), by microwave (89%), m.p.: 209–210 °C; IR (KBr): νcm^{-1} 3214 (NH), 2225 (br, 2CN), 1713, 1685 (2CO); ^{1}H NMR (DMSO-d_6): δ = 1.30 (t, 3H, J = 7.2 Hz, CH_3CH_2), 2.51 (s, 3H, CH_3), 4.18 (s, 2H, CH_2), 4.27 (q, 2H, J = 7.2 Hz, CH_3CH_2) and 12.51 ppm (s, 1H, NH); MS (EI): m/z (%) 277 (M$^+$, 42.75), 278 (M$^+$+1, 8.12). Anal. calcd. for $C_{12}H_{11}N_3O_3S$ (277.30): C, 51.98; H, 4.00; N, 15.15; S, 11.56. Found: C, 52.05; H, 3.98; N, 15.19; S, 11.50.

*4.3. General procedure for the preparation of enamines **4a,b***

Mixtures of **2a**,**b** (5 mmol), N,N-dimethylformamide dimethylacetal (DMF-DMA) (0.6 mL, 5 mmol) in dioxane (20 mL) were stirred at reflux for 2 h. The separated solid product obtained on standing at room temperature was collected by filtration, washed by EtOH and recrystallized from dioxane to afford the corresponding enamines **4a**,**b**.

*4.3.1. (E)-2-cyano-N-(3-cyano-4-phenylthiophen-2-yl)-3-dimethylaminoacrylamide (**4a**)*

yellowish white crystals, yield: 93%, m.p.: 218–219 °C; IR (KBr): νcm^{-1} 3364 (NH), 2191 (br, 2CN), 1675 (CO); ^{1}H NMR (DMSO-d_6): δ = 3.28 (s, 3H, CH_3), 3.33 (s, 3H, CH_3), 7.26 (s, 1H, thiophene H-5), 7.41 (t, J = 7.6 Hz, 1H, Ar–H), 7.49 (t, J = 7.6 Hz, 2H, Ar–H), 7.60 (d, J = 7.6 Hz, 2H, Ar–H), 7.94 (s. 1H, olefinic C*H*) and 10.40 ppm (s, 1H,

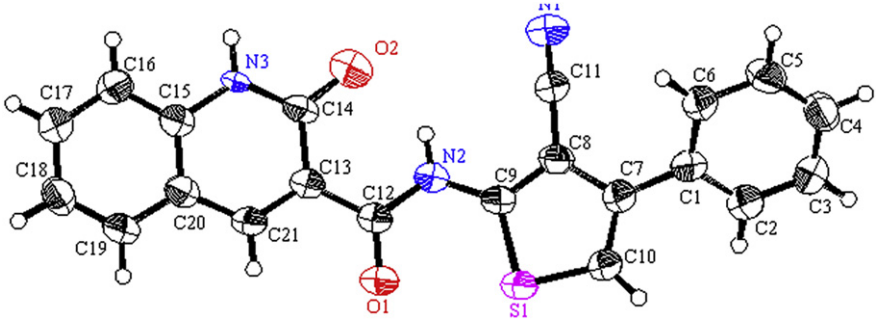


Fig. 6. ORTEP plot of the X-ray crystallographic data determined for **24a**. Crystallographic data have been deposited in the Cambridge crystallographic data Center as supplementary publication number CCDC 845670 [28].

Table 2
Selected bond lengths and bond angles for **24a**.

Bond	Bond length(Å)	Bond	Bond angle(°)
N3–C15	1.388	C14–N3–C15	123.0
N3–C14	1.367	N3–C14–C13	117.6
O2–C14	1.273	N3–C15–C20	119.9
C13–C14	1.465	C13–C21–C20	122.2
C13–C21	1.354	C14–C13–C21	118.9
S1–C10	1.708	C15–C20–C21	118.3
S1–C9	1.721	C9–S1–C10	90.9
O1–C12	1.226	C9–N2–C12	124.7

NH); ^{13}C NMR (DMSO-d_6): δ = 38.36(CH_3), 47.25(CH_3), 69.27 (C-2), 93.20 (thiophene C-3), 114.96 (CN), 115.52 (CN), 118.54, 127.25, 128.11, 128.77, 133.65, 137.49, 151.75, 157.40 and 163.39 ppm (Ar–C, olefinic C and CO); MS (EI): *m*/*z* (%) 322 (M^+, 29.00), 323 (M^++1, 6.95). Anal. calcd. for $C_{17}H_{14}N_4OS$ (322.39): C, 63.34; H, 4.38; N, 17.38; S, 9.95. Found: C, 63.30; H, 4.37; N, 17.35; S, 9.99.

*4.3.2. Crystallographic analysis for **4a***

The crystals were mounted on a glass fiber. All measurements were performed on a Rigaku R-AXIS RAPID diffractometer using filtered Mo-Kα radiation. The data were collected at a temperature of 20 ± 1 °C to a maximum 2θ value of 55.0° using the ω scanning technique. The structure was solved by charge flipping method and expanded using Fourier techniques. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model.

4.3.3. Crystal data

$C_{17}H_{14}N_4OS$, M = 322.39, triclinic, a = 7.451(8) Å, b = 10.282(1) Å, c = 11.389(1) Å, V = 767.9(2) Å^3, α = 102.987(8)°, β = 100.048(7)°, γ = 110.056(8)°, space group: P-1, Z = 2, D_{calc} = 1.394 g cm^{-3}, No. of reflection measured 3499, $2\theta_{max}$ = 54.9°, $R1$ = 0.041. Fig. 2 illustrates the structure as determined. Full data can be obtained on request from the CCDC [24].

*4.3.4. (E)-N-[4-(4-Chlorophenyl)-3-cyanothiophen-2-yl]-2-cyano-3-dimethylaminoacrylamide (**4b**)*

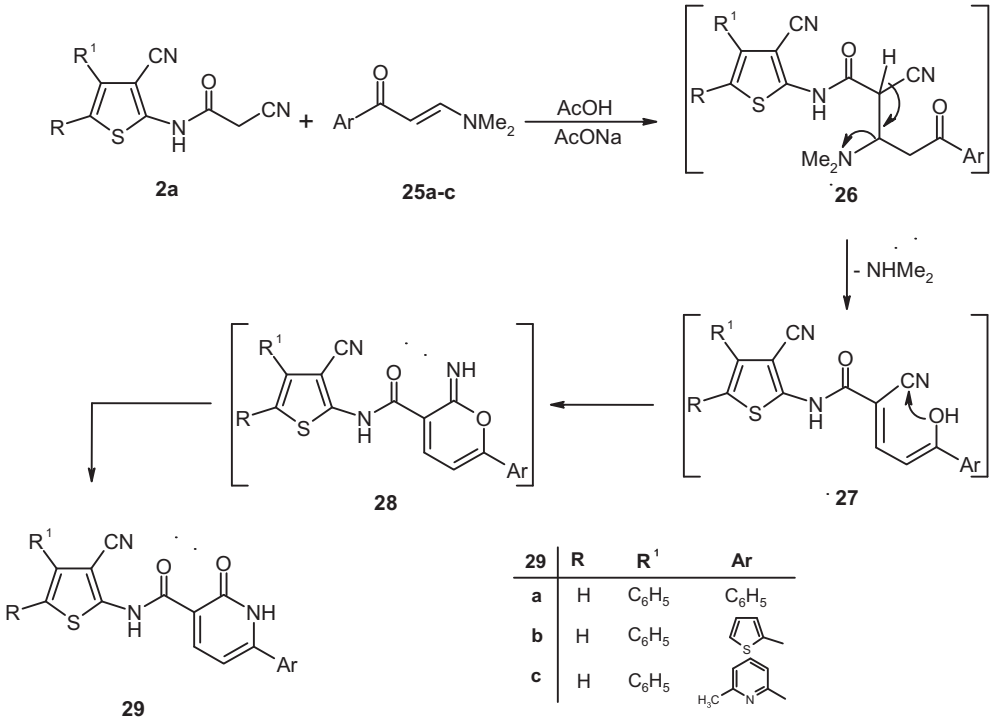
yellowish white crystals, yield: 91%, m.p.: 266–267 °C; IR (KBr): νcm^{-1} 3379 (NH), 2205, 2190 (2CN), 1672 (CO); ^{1}H NMR (DMSO-d_6): δ = 3.28 (s, 3H, CH_3), 3.33 (s, 3H, CH_3), 7.30 (s, 1H, thiophene H-5), 7.55 (d, J = 7.6 Hz, 2H, Ar–H), 7.62 (d, J = 7.6 Hz, 2H, Ar–H), 7.94 (s. 1H, olefinic C*H*) and 10.46 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 38.45(CH_3), 47.35(CH_3), 69.30 (C-2), 93.01 (thiophene C-3), 114.92 (CN), 116.20 (CN), 118.63, 128.91, 129.11, 132.54, 133.00, 136.19, 152.02, 157.53 and 163.62 ppm (Ar–C, olefinic C and CO); MS (EI): *m*/*z* (%) 356 (M^+, 14.20), 357 (M^++1, 4.15). Anal. calcd. for $C_{17}H_{13}ClN_4OS$ (356.84): C, 57.22; H, 3.67; N, 15.70; S, 8.99. Found: C, 57.18; H, 3.65; N, 15.76; S, 9.03.

*4.4. General procedure for the preparation of 5-amiopyrazole **5a,b***

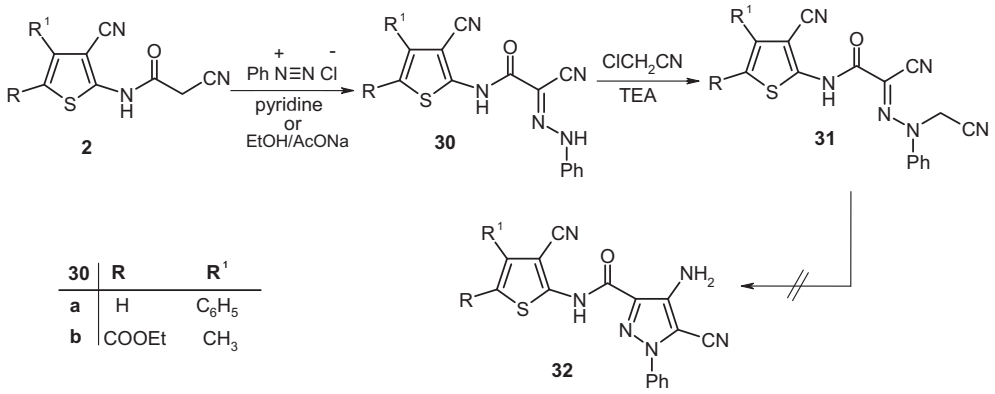
Solutions of the enaminonitrile **4a**,**b** (5 mmol) and hydrazine hydrate (80%, 0.35 mL) in dioxane (20 mL) were stirred at reflux for 5 h. The reaction mixtures were cooled to room temperature and poured into ice cold water containing a few drops of hydrochloric acid (2N). The formed solid products were collected by filtration, washed with water then ethanol and crystallized from EtOH/dioxane (2:1) mixture to afford the corresponding 5-amiopyrazole **5a**,**b**.

*4.4.1. 5-Amino-N-(3-cyano-4-phenylthiophen-2-yl)-1H-pyrazole-4-carboxamide (**5a**)*

yellow crystals, yield: 70%, m.p.: 244–245 °C; IR (KBr): νcm^{-1} 3465, 3362, 3257, 3198 (NH_2 and 2NH), 2215 (CN), 1649 (CO); ^{1}H NMR (DMSO-d_6): δ = 6.30 (br, 2H, NH_2), 7.28 (s, 1H, thiophene H-5), 7.42 (t, J = 7.2 Hz, 1H, Ar–H), 7.50 (t, J = 7.2 Hz, 2H, Ar–H), 7.61 (d, J = 7.2 Hz, 2H, Ar–H), 7.94 (s, 1H, pyrazole H-3), 11.03 (s, 1H, NH) and 11.94 ppm (s, 1H, NH); MS (EI): *m*/*z* (%) 309 (M^+, 14.80), 310 (M^++1, 3.95). Anal. calcd. for $C_{15}H_{11}N_5OS$ (309.35): C, 58.24; H, 3.58; N, 22.64; S, 10.36. Found: C, 58.18; H, 3.61; N, 22.58; S, 10.40.



Scheme 6. Reaction of cyanoacetamides **2a** with the enaminones **25a–c**.



Scheme 7. Reaction of cyanoacetamides **2** with bezenediazonium chloride.

*4.4.2. 5-Amino-N-[4-(4-Chlorophenyl)-3-cyanothiophen-2-yl]-1H-pyrazole-4-carboxamide (**5b**)*

pale yellow crystals, yield: 68%, m.p.: 250–252 °C; IR (KBr): νcm^{-1} 3465, 3404, 3368, 3325 (NH_2 and 2NH), 2218 (CN), 1655 (CO); ^{1}H-NMR (DMSO-d_6): δ = 6.35 (br, 2H, NH_2), 7.33 (s, 1H, thiophene H-5), 7.56 (d, J = 7.2 Hz, 2H, Ar–-H), 7.63 (d, J = 7.2 Hz, 2H, Ar–H), 8.03 (s, 1H, pyrazole H-3), 11.08 (s, 1H, NH) and 12.00 ppm (s, 1H, NH); MS (EI): *m/z* (%) 343 (M^+, 13.18), 344 (M^++1, 3.20). Anal. calcd. for $C_{15}H_{10}ClN_5OS$ (343.80): C, 52.41; H, 2.93; N, 20.37; S, 9.33. Found: C, 52.49; H, 2.98; N, 20.44; S, 9.30.

*4.5. General procedure for synthesis of pyrido[1,2-a]thieno[3,2-e] pyrimidine derivatives **12a–o***

Independent mixtures of cyanoacetamides **2a-c** (5 mmol) and the appropriate arylidenmalononitrile **6** (5 mmol) in ethanol (30 mL) containing few drops of piperidine (5 drops) were stirred at reflux for 1 h then, the reaction mixtures were cooled to room temperature. The solid which formed was collected by filtration, washed with hot ethanol, and recrystallized from the indicated solvent to afford **12a-o** respectively, as pure substances.

*4.5.1. 9-Imino-4-oxo-3,7-diphenyl-5,9-dihydro-4H-pyrido[1,2-a] thieno[3,2-e]pyrimidine-6,8-dicarbo- nitrile (**12a**)*

Recrystallized from an EtOH/DMF (1:3) mixture as pale yellow crystals, yield: 94%, m.p.: above 300 °C; IR (KBr): νcm^{-1} 3445, 3307 (2NH), 2214 (br, 2CN), 1651, 1628 (CO and C=N); ^{1}H NMR (DMSO-d_6 at 120 °C): δ = 6.01 (s, 1H, thiophene H-2), 7.54–7.56 (m, 2H, Ar–H), 7.57–7.60 (m, 8H, Ar–H), 7.74 (s, 1H, NH) and 8.92 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6 at 120 °C): δ = 85.35, 86.58, 113.85, 115.12 (CN), 115.15 (CN), 125.78, 127.61, 127.91, 128.50, 128.61, 128.73, 129.55, 133.42, 133.62, 134.30, 144.81, 150.67, 156.37, 157.15 and 158.81 ppm (Ar–C and CO); MS (EI): *m/z* (%) 419 (M^+, 100), 420 (M^++1, 29.75). Anal. calcd. for $C_{24}H_{13}N_5OS$ (419.47): C, 68.72; H, 3.12; N, 16.70; S, 7.64. Found: C, 68.76; H, 3.09; N, 16.75; S, 7.66.

*4.5.2. 9-Imino-4-oxo-3-phenyl-7-p-tolyl-5,9-dihydro-4H-pyrido [1,2-a]thieno[3,2-e]pyrimidine-6,8-di- carbonitrile (**12b**)*

Recrystallized from an EtOH/DMF (1:4) mixture as pale yellow crystals, yield: 91%, m.p.: above 300 °C; IR (KBr): νcm^{-1} 3447, 3301 (2NH), 2214 (br, 2CN), 1653, 1629 (CO and C=N); ^{1}H NMR (DMSO-d_6 at 120 °C): δ = 2.45 (s, 3H, CH_3), 6.12 (s, 1H, thiophene H-2), 7.40 (d, J = 7.6 Hz, 2H, Ar–H), 7.49 (d, J = 7.6 Hz, 2H, Ar–H), 7.54–7.60

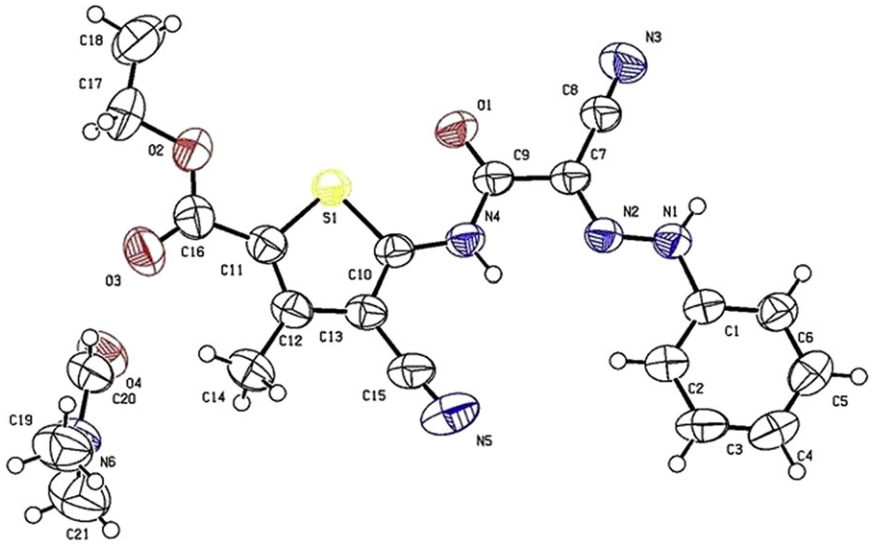


Fig. 7. ORTEP plot of the x-ray crystallographic data determined for **30b** containing one DMF molecule. Crystallographic data have been deposited in the Cambridge crystallographic data Center as supplementary publication number CCDC 841962 [31].

Table 3
Selected bond lengths and bond angles for **30b**.

Bond	Bond length(Å)	Bond	Bond angle(°)
C12–C13	1.423	C10–S1–C11	90.4
C12–C11	1.371	C12–C11–S1	113.4
C13–C10	1.364	C13–C10–S1	112.2
S1–C10	1.715	C10–C13–C12	113.8
S1–C11	1.733	C11–C12–C13	110.2
N1–N2	1.310	C7–N2–N1	122.6

(m, 5H, Ar–H), 7.73 (s, 1H, NH) and 9.03 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6 at 120 °C): δ = 20.30 (CH_3), 85.34, 86.57, 113.83, 115.24 (CN), 115.28 (CN), 125.76, 127.65, 128.49, 128.50, 128.61, 128.75, 131.38, 133.45, 133.62, 139.54, 144.80, 150.69, 156.35, 157.18 and 158.88 ppm (Ar–C and CO); MS (EI): *m/z* (%) 433 (M^+, 100), 434 (M^++1, 26.80). Anal. calcd. for $C_{25}H_{15}N_5OS$ (433.50): C, 69.27; H, 3.49; N, 16.16; S, 7.40. Found: C, 69.21; H, 3.52; N, 16.20; S, 7.37.

*4.5.3. 9-Imino-7-(4-methoxyphenyl)-4-oxo-3-phenyl-5,9-dihydro-4H-pyrido[1,2-a]thieno[3,2-e]pyrim- idine-6,8-dicarbonitrile (**12c**)*

Recrystallized from an EtOH/DMF (1:2) mixture as yellow crystals, yield: 88%, m.p.: above 300 °C; IR (KBr): νcm^{-1} 3444, 3301 (2NH), 2222, 2210 (2CN), 1648, 1630 (CO and C=N); ^{1}H NMR (DMSO-d_6): δ = 3.87 (s, 3H, CH_3), 6.17 (s, 1H, thiophene H-2), 7.15 (d, *J* = 7.6 Hz, 2H, Ar–H), 7.52 (d, *J* = 7.6 Hz, 2H, Ar–H), 7.55–7.59 (m, 5H, Ar–H), 7.77 (s, 1H, NH) and 9.36 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 55.35 (CH_3), 85.58, 86.57, 113.97, 114.02, 116.41 (CN), 116.44 (CN), 126.37, 126.47, 129.01, 129.19, 129.28, 130.19, 133.80, 134.03, 145.23, 151.16, 156.66, 157.89, 158.76 and 160.83 ppm (Ar–C and CO); MS (EI): *m/z* (%) 449 (M^+, 100), 450 (M^++1, 30.30). Anal. calcd. for $C_{25}H_{15}N_5O_2S$ (449.49): C, 66.80; H, 3.36; N, 15.58; S, 7.13. Found: C, 66.77; H, 3.41; N, 15.52; S, 7.17.

*4.5.4. 9-Imino-7-(4-nitrophenyl)-4-oxo-3-phenyl-5,9-dihydro-4H-pyrido[1,2-a]thieno[3,2-e]pyramid- ine-6,8-dicarbonitrile (**12d**)*

Recrystallized from an EtOH/DMF (1:2) mixture as yellow crystals, yield: 84%, m.p.: above 300 °C; IR (KBr): νcm^{-1} 3435, 3302 (2NH), 2214 (br, 2CN), 1658, 1631 (CO and C=N); ^{1}H NMR (DMSO-d_6): δ = 6.31 (s, 1H, thiophene H-2), 7.54–7.57 (m, 5H, Ar–H), 7.80 (s, 1H, NH), 7.92 (d, *J* = 7.6 Hz, 2H, Ar–H), 8.47 (d, *J* = 7.6 Hz, 2H, Ar–H) and 9.51 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 85.48, 86.41, 114.17, 115.80 (CN), 115.87 (CN), 123.89, 126.88, 129.07, 129.22, 129.31, 130.09, 133.66, 134.15, 140.80, 148.54, 150.90, 156.74, 156.95, 157.59 and 157.70 ppm (Ar–C and CO); MS (EI): *m/z* (%) 464 (M^+, 100), 465 (M^++1, 31.70). Anal. calcd. for $C_{24}H_{12}N_6O_3S$ (464.47): C, 62.06; H, 2.60; N, 18.09; S, 6.90. Found: C, 62.10; H, 2.55; N, 18.07; S, 6.94.

*4.5.5. 7-(4-Chlorophenyl)-9-imino-4-oxo-3-phenyl-5,9-dihydro-4H-pyrido[1,2-a]thieno[3,2-e]pyramid- ine-6,8-dicarbonitrile (**12e**)*

Recrystallized from an EtOH/DMF (1:3) mixture as yellow crystals, yield: 90%, m.p.: above 300 °C; IR (KBr): νcm^{-1} 3443, 3304 (2NH), 2214 (br, 2CN), 1650, 1628 (CO and C=N); ^{1}H NMR (DMSO-d_6): δ = 6.28 (s, 1H, thiophene H-2), 7.53–7.61 (m, 5H, Ar–H), 7.63 (d, *J* = 7.6 Hz, 2H, Ar–H), 7.71 (d, *J* = 7.6 Hz, 2H, Ar–H), 7.81 (s, 1H, NH) and 9.45 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 85.22, 86.34, 113.77, 115.05 (CN), 115.18 (CN), 125.94, 128.17, 128.51, 128.61, 128.73, 129.61, 132.97, 133.31, 133.60, 134.72, 144.72, 150.56, 156.31, 157.07 and 157.46 ppm (Ar–C and CO); MS (EI): *m/z* (%) 453 (M^+, 100), 454 (M^++1, 33.54). Anal. calcd. for $C_{24}H_{12}ClN_5OS$ (453.91): C, 63.51; H, 2.66; N, 15.43; S, 7.06. Found: C, 63.55; H, 2.60; N, 15.44; S, 7.11.

*4.5.6. 9-Imino-4-oxo-3-phenyl-7-(thiophen-2-yl)-5,9-dihydro-4H-pyrido[1,2-a]thieno[3,2-e]pyrimidine -6,8-dicarbonitrile (**12f**)*

Recrystallized from an EtOH/DMF (1:2) mixture as pale yellow crystals, yield: 81%, m.p.: above 300 °C; IR (KBr): νcm^{-1} 3474, 3362 (2NH), 2213 (br, 2CN), 1651, 1610 (CO and C=N); ^{1}H NMR (DMSO-d_6 at 90 °C): δ = 6.22 (s, 1H, H-2), 7.32 (t, *J* = 5.4 Hz, 1H, thiophene H), 7.52–7.59 (m, 5H, Ar–H), 7.63 (d, *J* = 5.4 Hz, 1H, thiophene H), 7.79 (s, 1H, NH), 8.00 (d, *J* = 5.4 Hz, 1H, thiophene H) and 9.40 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6 at 90 °C): δ = 85.55, 86.57, 113.99, 115.45 (CN), 115.53 (CN), 126.04, 127.19, 128.70, 128.80, 128.88, 129.99, 130.55, 133.25, 133.51, 133.79, 144.99, 150.87, 151.17, 156.53 and 157.30 ppm (Ar–C and CO); MS (EI): *m/z* (%) 425 (M^+, 100), 426 (M^++1, 26.95). Anal. calcd. for $C_{22}H_{11}N_5OS_2$ (425.49): C, 62.10; H, 2.61; N, 16.46; S, 15.07. Found: C, 62.03; H, 2.58; N, 16.50; S, 15.11.

*4.5.7. 3-(4-Chlorophenyl)-9-imino-4-oxo-7-phenyl-5,9-dihydro-4H-pyrido[1,2-a]thieno[3,2-e]pyramid- ine-6,8–dicarbonitrile (**12g**)*

Recrystallized from DMF as canary yellow crystals, yield: 89%, m.p.: above 300 °C; IR (KBr): νcm^{-1} 3442, 3301 (2NH), 2211 (br, 2CN), 1657, 1630 (CO and C=N); ^{1}H NMR (DMSO-d_6 at 85 °C): δ = 6.64 (s, 1H, thiophene H-2), 7.52–7.62 (m, 9H, Ar–H), 7.80 (s, 1H, NH) and 9.33 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6 at 85 °C): δ = 85.35, 86.47, 113.71, 115.33 (CN), 115.36 (CN), 126.42, 127.72, 128.05, 128.67, 129.69, 130.60, 132.19, 132.56, 133.56, 134.36, 145.07, 150.67, 156.32, 157.29 and 158.89 ppm (Ar–C and CO); MS (EI): *m/z* (%) 453 (M^+, 100), 454 (M^++1, 33.05). Anal. calcd. for $C_{24}H_{12}ClN_5OS$ (453.91): C, 63.51; H, 2.66; N, 15.43; S, 7.06. Found: C, 63.48; H, 2.70; N, 15.44; S, 7.00.

*4.5.8. 3-(4-Chlorophenyl)-9-imino-4-oxo-7-p-tolyl-5,9-dihydro-4H-pyrido[1,2-a]thieno[3,2-e]pyramid- ine-6,8–dicarbonitrile (**12h**)*

Recrystallized from an EtOH/DMF (1:3) mixture as yellow crystals, yield: 85%, m.p.: above 300 °C; IR (KBr): νcm^{-1} 3447, 3299

Table 4
Inhibition zone diameter of the tested chemicals that showed strong antimicrobial activities against the tested microorganisms.

Compound no.	Inhibition zone diameter (Nearest mm) Average(±standard deviation)						
	E.coli	*P . aeruginosa*	*S. marcescens*	*B.subtilis*	*S. aureus*	*C. albicans*	*S. cerevisiae*
1a	10.6(1.1)	10.6(1.1)	11.3(1.1)	14.6(1.1)	21(2)	18(2)	16(0)
1b	4(0)	5(1.1)	0	11(0)	6.6 (1.1)	2.6 (1.1)	7 (1.1)
4a	4(0)	10.8(0)	3.3(1.1)	1.6(0.5)	0	4(4)	1.3(1.1)
12c	3.3(1.1)	14(0)	2(0)	2(0)	0	0	0
12f	4(0)	11.3(0)	0	3.3(2.3)	0	0	0
12i	4(0)	13.1(1.1)	4(0)	2(0)	0	0	0
12 l	4(0)	10.6(1.1)	2.6(1.1)	2(0)	0	12(0)	0
19a	4(0)	9(2)	6.6(1.1)	2(0)	0	2.6(4)	1.3(1.1)
19b	2(0)	8(0)	3.3(1.1)	6(2)	2.6(2.3)	0	0
24a	8.6(2.3)	10(3.4)	6(0)	11(0)	12(0)	16(0)	14(2)
24b	8(0)	11(0.5)	–	10.5(0.15)	8(0.06)	–	–
30b	0	6(0)	2.6(1.1)	13(0.2)	14 (0.5)	0	0

Table 5
Inhibition zone diameter of the tested chemicals that showed moderate to week antimicrobial activities against the tested microorganisms.

Chemical name	Inhibition zone diameter (Nearest mm) Average(±standard deviation)						
	E.coli	*P.aeuroginas*	*S. marcescens*	*B.subtilus*	*S. aureus*	*C.albicans*	*S. cerevisiae*
2a	4(0)	4(0)	2(0)	2(0)	0	4(0)	2.6(1.1)
2b	4(0)	4(0)	1.3 (1.1)	4.6 (1.1)	0	0	3 (1.3)
2c	7(0.02)	–	–	7(0.02)	–	0	0
4b	4(0)	4(0)	7(1.1)	1(0)	0	0	1.3(1.1)
5a	2.6(2.3)	6.6(1.1)	0.7(1)	2(0)	0	4(2)	2.6(2)
5b	0	3.3(1)	2(0)	7.3(1.1)	2(0)	6.6(1.1)	4(0)
12a	6.3(1.1)	4(0)	4.6(1.1)	4(0)	0	2(0)	2.6(1.1)
12b	2.6(1.1)	6(0)	3.3(1)	2(0)	0	0	0.7(1.1)
12d	4(0)	6.6(1.1)	4.6(1.1)	2(0)	0	0	0
12e	4.6(1.1)	4.3(2.3)	3.3(1.1)	1.6(0.5)	0	0	1.3(1.1)
12g	4(0)	6(0)	4(0)	2(0)	0	0	4(0)
12h	3.3(1.1)	6(0)	4(0)	2(0)	0	0	2(0)
12j	5.3(1.1)	6(0)	4(2)	4(0)	0	2.6(2)	1.3(1.1)
12k	4(0)	7(0)	2.6(1.1)	3.3(1.1)	0	0	0.7(1)
12o	8(0.1)	6(0)	4.6(2.3)	2(0)	0	0	0
21a	2(0)	4(0)	7.3(1.1)	0	0	0	0
21b	2(0)	6(0)	2(0)	3.6(0.5)	0	0	0
29a	3.3(1.1)	5.3(1.1)	2(0)	1.6(0.5)	0	0	1.3(1)
29b	2(0)	4(2)	2(0)	2(0)	0	0	2(0)
29c	0	4(2)	0	0	0	0	0
DMSO (solvent)[a]	0	0	0	0	0	0	0
Penicillin[b]	8.6(1.1)	30(0)	7(1.1)	4.6(1.1)	40(0)	–	–
Cycloheximide[c]	–	–	–	–	–	0	40(0)

– not tested.
[a] DMSO = Dimethyl sulfoxide.
[b] Antibacterial drug.
[c] Antifungal drug.

(2NH), 2212 (br, 2CN), 1643, 1629 (CO and C=N); ^{1}H NMR (DMSO-d_6 at 110 °C): δ = 2.33 (s, 3H, CH_3), 6.63 (s, 1H, thiophene H-2), 7.42 (d, J = 7.2 Hz, 2H, Ar–H), 7.49 (d, J = 7.2 Hz, 2H, Ar–H), 7.54 (d, J = 7.6 Hz, 2H, Ar–H), 7.61 (d, J = 7.6 Hz, 2H, Ar–H), 7.80 (s, 1H, NH) and 9.30 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6 at 110 °C): δ = 20.33 (CH_3), 85.29, 86.46, 113.67, 115.35 (CN), 115.39 (CN), 126.29, 127.66, 128.53, 128.62, 130.54, 131.41, 132.17, 132.50, 133.55, 139.57, 145.03, 150.58, 156.27, 157.25 and 158.94 ppm (Ar–C and CO); MS (EI): m/z (%) 467 (M^+, 100), 468 (M^++1, 32.80). Anal. calcd. for $C_{25}H_{14}ClN_5OS$ (467.94): C, 64.17; H, 3.02; N, 14.97; S, 6.85. Found: C, 64.20; H, 2.98; N, 14.99; S, 6.91.

4.5.9. 9-Imino-7-(4-methoxyphenyl)-4-oxo-3-phenyl-5,9-dihydro-4H-pyrido[1,2-a]thieno[3,2-e]pyri- midine-6,8-dicarbonitrile (**12i**)

Recrystallized from an EtOH/DMF (1:2) mixture as pale yellow crystals, yield: 89%, m.p.: above 300 °C; IR (KBr): νcm^{-1} 3475, 3373 (2NH), 2222 (br, 2CN), 1664, 1623 (CO and C=N); ^{1}H NMR (DMSO-d_6): δ = 3.87 (s, 3H, CH_3), 6.58 (s, 1H, thiophene H-2), 7.16 (d, J = 7.6 Hz, 2H, Ar–H), 7.52–7.62 (m, 6H, Ar–H), 7.78 (s, 1H, NH) and 9.27 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 55.94 (CH_3), 86.26, 87.38, 114.62, 116.49 (CN), 116.54 (CN), 127.26, 129.61, 130.53, 131.14, 131.53, 132.52, 133.17, 133.49, 134.51, 146.01, 151.67, 157.24, 158.29, 159.59 and 161.52 ppm (Ar–C and CO); MS (EI): m/z (%) 483 (M^+, 100), 484 (M^++1, 27.00). Anal. calcd. for $C_{25}H_{14}ClN_5O_2S$ (483.94): C, 62.05; H, 2.92; N, 14.47; S, 6.63. Found: C, 62.03; H, 2.89; N, 14.53; S, 6.60.

4.5.10. 3-(4-Chlorophenyl)-9-imino-7-(4-nitrophenyl)-4-oxo-3-phenyl-5,9-dihydro-4H-pyrido[1,2-a]- thieno[3,2-e]pyrimidine-6,8-dicarbonitrile (**12j**)

Recrystallized from DMSO as yellow crystals, yield: 86%, m.p.: above 300 °C; IR (KBr): νcm^{-1} 3435, 3310 (2NH), 2219 (br, 2CN), 1654, 1630 (CO and C=N); ^{1}H NMR (DMSO-d_6): δ = 6.78 (s, 1H, thiophene H-2), 7.53 (d, J = 8.0 Hz, 2H, Ar–H), 7.61 (d, J = 8.0 Hz, 2H, Ar–H), 7.83 (s, 1H, NH) 7.89 (d, J = 8.2 Hz, 2H, Ar–H), 8.46 (d, J = 8.2 Hz, 2H, Ar–H), and 9.41 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 85.36, 86.16, 113.96, 115.86 (CN), 115.92 (CN), 123.90, 127.21, 129.12, 130.02, 131.05, 132.39, 133.09, 133.75, 140.84, 145.43, 148.53, 150.88, 156.63, 157.00, and 157.69 ppm (Ar–C and CO); MS (EI): m/z (%) 498 (M^+, 100), 499 (M^++1, 29.15). Anal. calcd. for $C_{24}H_{11}ClN_6O_3S$ (498.91): C, 57.78; H, 2.22; N, 16.84; S, 6.43. Found: C, 57.75; H, *2.18; N, 16.79; S, 6.37.*

4.5.11. Crystallographic analysis for **12j**

The crystals were mounted on a glass fiber. All measurements were performed on a Rigaku R-AXIS RAPID diffractometer using filtered Mo-Kα radiation. The data were collected at a temperature of 20 ± 1 °C to a maximum 2θ value of 55.0° using the ω scanning technique. The structure was solved by charge flipping method and expanded using Fourier techniques. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model.

4.5.12. Crystal data

$C_{24}H_{11}ClN_6O_3S$, M = 498.91, triclinic, a = 9.8993(7) Å, b = 15.558(2) Å, c = 17.974(2) Å, V = 2661.2(4) Å^3, α = 91.591(7)°, β = 102.724(8)°, γ = 99.035(7)°, space group: P-1, Z = 2, D_{calc} = 1.425 g cm^{-3}, No. of reflection measured 11953, $2\theta_{max}$ = 54.9°, $R1$ = 0.088. Fig. 3 illustrates the structure as determined. Full data can be obtained on request from the CCDC [25].

4.5.13. 3,7-Bis(4-chlorophenyl)-9-imino-4-oxo-5,9-dihydro-4H-pyrido[1,2-a]thieno[3,2-e]pyrimidine-6,8-dicarbonitrile (**12k**)

Recrystallized from an EtOH/DMF (1:2) mixture as yellow crystals, yield: 88%, m.p.: above 300 °C; IR (KBr): νcm^{-1} 3439, 3301 (2NH), 2215 (br, 2CN), 1658, 1631 (CO and C=N); ^{1}H NMR (DMSO-d_6): δ = 6.74 (s, 1H, thiophene H-2), 7.54 (d, J = 8.0 Hz, 2H, Ar–H), 7.61–7.64 (m, 4H, Ar–H), 7.71 (d, J = 8.0 Hz, 2H, Ar–H), 7.82 (s, 1H, NH) and 9.38 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 85.56, 86.41, 113.92, 116.16 (CN), 116.19 (CN), 127.15, 128.88, 129.15, 130.33, 131.10, 132.48, 133.08, 133.40, 133.77, 135.30, 145.45, 150.95, 156.61, 157.81 and 157.91 ppm (Ar–C and CO); MS (EI): m/z (%) 487 (M^+,

100), 488 (M^++1, 34.70). Anal. calcd. for $C_{24}H_{11}Cl_2N_5OS$ (488.36): C, 59.03; H, 2.27; N, 14.34; S, 6.57. Found: C, 58.99; H, 2.25; N, 14.40; S, 6.65.

4.5.14. 3-(4-Chlorophenyl)-9-imino-4-oxo-7-(thiophen-2-yl)-5,9-dihydro-4H-pyrido[1,2-a]thieno[3,2-e]pyrimidine-6,8-dicarbonitrile (**12l**)

Recrystallized from an EtOH/DMF (1:2) mixture as pale brown crystals, yield: 85%, m.p.: above 300 °C; IR (KBr): νcm^{-1} 3444, 3303 (2NH), 2209 (br, 2CN), 1649, 1628 (CO and C=N); ^{1}H NMR (DMSO-d_6 at 90 °C): δ = 6.45 (s, 1H, H-2), 7.32 (t, J = 5.0 Hz, 1H, thiophene H), 7.63 (d, J = 7.6 Hz, 2H, Ar–H), 7.59–7.63 (m, 3H, 2Ar–H and one thiophene H), 7.78 (s, 1H, NH), 7.95 (d, J = 5.0 Hz, 1H, thiophene H) and 9.16 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6 at 90 °C): δ = 85.41, 86.31, 113.73, 115.64 (CN), 115.72 (CN), 126.64, 127.28, 128.78, 130.16, 130.66, 130.69, 132.26, 132.66, 133.22, 133.60, 145.17, 150.80, 151.08, 156.38 and 157.39 ppm (Ar-C and CO); MS (EI): m/z (%) 459 (M^+, 100), 460 (M^++1, 31.60). Anal. calcd. for $C_{22}H_{10}ClN_5OS_2$ (459.94): C, 57.45; H, 2.19; N, 15.23; S, 13.94. Found: C, 57.49; H, 2.21; N, 15.17; S, 13.98.

4.5.15. 6.8-Dicyano-9-imino-3-methyl-4-oxo-7-phenyl-5,9-dihydro-4H-pyrido[1,2-a]thieno[3,2-e]pyri- midine-2-carboxylic acid ethyl ester (**12m**)

Recrystallized from DMF as yellow crystals, yield: 90%, m.p.: above 300 °C; IR (KBr): νcm^{-1} 3489, 3313 (2NH), 2225 (br, 2CN), 1718 (CO ester), 1661, 1643 (CO and C=N); ^{1}H NMR (DMSO-d_6): δ = 1.35 (t, 3H, J = 7.2 Hz, CH_3CH_2), 2.92 (s, 3H, CH_3), 4.36 (q, 2H, J = 7.2 Hz, CH_3CH_2), 7.58–7.62 (m, 5H, Ar–H), 8.19 (s, 1H, NH) and 9.55 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 13.99 (CH_3), 14.34 (CH_3), 61.59 (CH_2), 85.64, 86.67, 115.73 (CN), 115.78 (CN), 116.95, 125.16, 128.32, 128.61, 130.49, 134.30, 138.22, 145.57, 151.06, 156.92, 157.70, 159.70, and 161.54 ppm (Ar-C and CO); MS (EI): m/z (%) 429 (M^+, 100), 430 (M^++1, 24.89). Anal. calcd. for $C_{22}H_{15}N_5O_3S$ (429.46): C, 61.53; H, 3.52; N, 16.31; S, 7.47. Found: C, 61.59; H, 3.55; N, 16.37; S, 7.49.

4.5.16. 6.8-Dicyano-9-imino-3-methyl-4-oxo-7-p-tolyl-5,9-dihydro-4H-pyrido[1,2-a]thieno[3,2-e]pyri- midine-2-carboxylic acid ethyl ester (**12n**)

Recrystallized from DMF as yellow crystals, yield: 81%, above 300 °C; IR (KBr): νcm^{-1} 3433, 3272 (2NH), 2220 (br, 2CN), 1704 (CO ester), 1657, 1639 (CO and C=N); ^{1}H NMR (DMSO-d_6): δ = 1.36 (t, 3H, J = 7.2 Hz, CH_3CH_2), 2.43 (s, 3H, CH_3), 2.95 (s, 3H, CH_3), 4.37 (q, 2H, J = 7.2 Hz, CH_3CH_2), 7.41 (d, J = 8.0 Hz, 2H, Ar–H), 7.47 (d, J = 8.0 Hz, 2H, Ar–H), 8.20 (s, 1H, NH) and 9.53 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 13.50 (CH_3), 13.87 (CH_3), 20.39 (CH_3), 60.95 (CH_2), 85.30, 86.53, 115.17 (CN), 115.19 (CN), 116.78, 125.58, 127.67, 128.57, 131.30, 137.31, 139.72, 145.75, 150.95, 156.99, 157.42, 159.51 and 161.37 ppm (Ar-C and CO); MS (EI): m/z (%) 443 (M^+, 100), 444 (M^++1, 27.80). Anal. calcd. for $C_{23}H_{17}N_5O_3S$ (433.49): C, 62.29; H, 3.86; N, 15.79; S, 7.23. Found: 62.33; H, 3.90; N, 15.84; S, 7.20.

4.5.17. 7-(4-Chlorophenyl)-6.8-dicyano-9-imino-3-methyl-4-oxo-5,9-dihydro-4H-pyrido[1,2-a]thieno- [3,2-e]pyrimidine-2-carboxylic acid ethyl ester (**12o**)

Recrystallized from DMF as yellow crystals, yield: 85%, above 300 °C; IR (KBr): νcm^{-1} 3490, 3337 (2NH), 2220 (br, 2CN), 1711 (CO ester), 1659, 1640 (CO and C=N); ^{1}H NMR (DMSO-d_6): δ = 1.34 (t, 3H, J = 7.2 Hz, CH_3CH_2), 2.84 (s, 3H, CH_3), 4.33 (q, 2H, J = 7.2 Hz, CH_3CH_2), 7.63 (d, J = 8.0 Hz, 2H, Ar–H), 7.70 (d, J = 8.0 Hz, 2H, Ar–H), 8.21 (s, 1H, NH) and 9.60 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 14.04 (CH_3), 14.40 (CH_3), 61.51 (CH_2), 85.56, 86.55, 115.80 (CN), 115.84 (CN), 117.15, 125.42, 128.84, 130.17, 133.21, 135.33, 138.17, 146.00, 151.30, 157.21, 157.88, 158.48 and 161.75 ppm (Ar-C and CO) not done; MS (EI): m/z (%) 463 (M^+, 100), 464 (M^++1, 25.08). Anal. calcd. for $C_{22}H_{14}ClN_5O_3S$ (463.91): C, 56.96; H, 3.04; N, 15.10; S, 6.91. Found: C, 57.02; H, 3.07; N, 15.14; S, 6.90.

4.5.18. Crystallographic analysis for **12o**

The crystals were mounted on a glass fiber. All measurements were performed on Bruker X8 Prospector. The data were collected at a temperature of 20 ± 1 °C to a maximum θ value of 66.61° using the ω scanning technique. The structure was solved by direct method using SHELXS-97 (Sheldrick, 2008) and refined by Full-matrix least-squares on F^2. The non-hydrogen atoms were refined anisotropically. Data were corrected for absorption effects using the multi-scan method (SADABS).

4.5.19. Crystal data

$C_{22}H_{14}ClN_5O_3S$, M = 463.91, monoclinic, a = 16.139(9) Å, b = 7.067(4) Å, c = 26.308(19) Å, V = 2964.8(3) Å^3, $\alpha = \gamma = 90.00°$, β = 98.862(7)°, space group: P 1 21/n 1, Z = 4, D_{calc} = 1.367 g cm^{-3}, No. of reflection measured 5415, θ_{max} = 25.34°, $R1$ = 0.0599. Fig. 4 illustrates the structure as determined. Full data can be obtained on request from the CCDC [26].

4.6. General procedure for synthesis of pyrido[1,2-a]thieno[3,2-e] pyrimidine derivatives **19a**,**b**

Independent mixtures of the enaminones **4a**,**b** (5 mmol) and the malononitrile (0.33 g, 5 mmol) in DMF (20 mL) containing few drops of piperidine (10 drops) were stirred at reflux for 4 h then, the reaction mixtures were cooled to room temperature. The solid which formed was collected by filtration, washed with hot ethanol, and recrystallized from DMF to afford **19a**,**b** respectively.

4.6.1. 9-Imino-4-oxo-3-phenyl-5,9-dihydro-4H-pyrido[1,2-a]thieno [3,2-e]pyrimidine-6,8-dicarbo- nitrile (**19a**)

yellowish white crystals, yield: 73%, above 300 °C; IR (KBr): νcm^{-1} 3433, 3302 (2NH), 2219 (br, 2CN), 1658, 1633 (CO and C=N); ^{1}H NMR (DMSO-d_6 at 110 °C): δ = 7.51–7.58 (m, 6H, 5Ar–H and thiophene H-2), 7.76 (s, 1H, H-7), 7.95 (s, 1H, NH) and 8.56 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6 at 110 °C): δ = 83.99, 85.41, 113.85, 115.50 (CN), 115.59 (CN), 125.70, 128.48, 128.57, 128.72, 133.41, 133.56, 144.87, 146.66, 151.12, 156.10 and 157.33 ppm (Ar-C and CO); MS (EI): m/z (%) 343 (M^+, 100), 344 (M^++1, 24.43). Anal. calcd. for $C_{18}H_9N_5OS$ (343.37): C, 62.96; H, 2.64; N, 20.40; S, 9.34. Found: C, 62.93; H, 2.70; N, 20.35; S, 9.33.

4.6.2. 3-(4-Chlorophenyl)-9-imino-4-oxo-5,9-dihydro-4H-pyrido [1,2-a]thieno[3,2-e]pyrimidine-6,8-dicarbonitrile (**19b**)

pale yellow crystals, yield: 75%, above 300 °C; IR (KBr): νcm^{-1} 3443, 3303 (2NH), 2219 (br, 2CN), 1661, 1634 (CO and C=N); ^{1}H NMR (DMSO-d_6 at 120 °C): δ = 6.66 (s, 1H, thiophene H-2), 7.52 (d, J = 8.0 Hz, 2H, Ar–H), 7.61 (d, J = 8.0 Hz, 2H, Ar–H), 7.78 (s, 1H, H-7), 8.57 (s, 1H, NH) and 9.20 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6 at 120 °C): δ = 84.98, 86.35, 114.75, 116.58 (CN), 116.67 (CN), 120.56, 127.26, 129.61, 131.52, 133.17, 133.45, 134.56, 138.38, 147.79, 157.06 and 158.42 ppm (Ar-C and CO); MS (EI): m/z (%) 377 (M^+, 100), 378 (M^++1, 33.90). Anal. calcd. for $C_{18}H_8ClN_5OS$ (377.81): C, 57.22; H, 2.13; N, 18.54; S, 8.49. Found: C, 57.18; H, 2.15; N, 18.48; S, 8.55.

4.7. General procedure for synthesis of the arylidene derivatives **21a**,**b**

4.7.1. Method A

A mixture of cyanoacetamides **2a**,**b** (5 mmol) and 2-(4-dimethylaminobenzylidene)malononitrile **20** (1.0 g, 5 mmol) was refluxed in EtOH (20 mL) in the presence of piperidine (5 drops) for

1 h. Then, the reaction mixture was allowed to cool to room temperature, the formed crude product was collected by filtration washed with water and recrystallized from the proper solvent.

4.7.2. Method B

A mixture of cyanoacetamides **2a**,**b** (5 mmol) and 4-dimethylaminobenzaldehyde (0.75 g, 5 mmol) in EtOH (20 mL) in the presence of piperidine (5 drops) were refluxed for 2 h with continuous stirring. The solid product which formed was collected by filtration, washed EtOH recrystallized from the proper solvent.

*4.7.3. (E)-2-cyano-N-(3-cyano-4-phenylthiophen-2-yl)-3-[4-(dimethylamino)phenyl]acrylamide (**21a**)*

Recrystallized from an EtOH/DMF (1:2) mixture as orange crystals, yield: 85%, m.p.: 278–280 °C; IR (KBr): νcm^{-1} 3373 (NH), 2212, 2198 (2CN), 1671 (CO); ^{1}H NMR (DMSO-d_6 at 90 °C): δ = 3.14 (s, 6H, 2CH$_3$), 6.87 (d, J = 8.6 Hz, 2H, Ar–H), 7.34 (s, 1H, thiophene H-5), 7.42 (t, J = 7.8 Hz, 1H, Ar–H), 7.49 (t, J = 7.8 Hz, 2H, Ar–H), 7.63 (d, J = 7.8 Hz, 2H, Ar–H), 7.96 (d, J = 8.6 Hz, 2H, Ar–H), 8.15 (s. 1H, olefinic C*H*) and 10.92 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6 at 90 °C): δ = 39.48 (2CH$_3$), 94.80 (C-2), 96.06 (thiophene C-3), 111.86, 114.38 (CN), 116.64 (CN), 117.42, 118.77, 127.37, 128.17, 128.70, 133.46, 133.68, 138.35, 155.65, 152.74, 153.90 and 161.58 ppm (Ar-C, olefinic C and CO); MS (EI): m/z (%) 398 (M$^+$, 20.15), 399 (M$^+$+1, 5.09). Anal. calcd. for $C_{23}H_{18}N_4OS$ (398.49): C, 69.33; H, 4.55; N, 14.06; S, 8.05. Found: C, 69.27; H, 4.59; N, 14.12; S, 8.01.

*4.7.4. Crystallographic analysis for **21a***

The crystals were mounted on a glass fiber. All measurements were performed on a Rigaku R-AXIS RAPID diffractometer using filtered Mo-Kα radiation. The data were collected at a temperature of 20 ± 1 °C to a maximum 2θ value of 55.0° using the ω scanning technique. The structure was solved by charge flipping method and expanded using Fourier techniques. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model.

4.7.5. Crystal data

$C_{23}H_{18}N_4OS$, M = 322.39, triclinic, a = 7.4449(4) Å, b = 12.8268(8) Å, c = 20.887(2) Å, V = 1960.4(2) Å^3, α = 85.238(6)°, β = 83.849(6)°, γ = 82.276(6)°, space group: P-1, Z = 4, D_{calc} = 1.35 g cm^{-3}, No. of reflection measured 8891, $2\theta_{max}$ = 54.9°, $R1$ = 0.060. Fig. 5 illustrates the structure as determined. Full data can be obtained on request from the CCDC [27].

*4.7.6. (E)-N-[4-(4-Chlorophenyl)-3-cyanothiophen-2-yl]-2-cyano-3-[4-(dimethylamino)phenyl]acryl- amide (**21b**)*

Recrystallized from an EtOH/DMF (1:2) mixture as orange crystals, yield: 79%, m.p.: 297–298 °C; IR (KBr): νcm^{-1} 3373 (NH), 2210, 2195 (2CN), 1673 (CO); ^{1}H NMR (DMSO-d_6): δ = 3.11 (s, 6H, 2CH$_3$), 6.87 (d, J = 9.0 Hz, 2H, Ar–H), 7.40 (s, 1H, thiophene H-5), 7.55 (d, J = 8.0 Hz, 2H, Ar–H), 7.65 (d, J = 8.0 Hz, 2H, Ar–H), 7.96 (d, J = 9.0 Hz, 2H, Ar–H), 8.15 (s. 1H, olefinic C*H*) and 11.16 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 39.09 (2CH$_3$), 94.85 (C-2), 96.93 (thiophene C-3), 111.46, 116.80 (CN), 117.21 (CN), 118.31, 127.41, 128.40, 128.74, 132.08, 132.79, 133.11, 135.10, 136.52, 152.46, 153.46 and 161.42 ppm (Ar-C, olefinic C and CO); MS (EI): m/z (%) 432 (M$^+$, 20.15), 433 (M$^+$+1, 4.09). Anal. calcd. for $C_{23}H_{17}ClN_4OS$ (432.94): C, 63.81; H, 3.96; N, 12.94; S, 7.41. Found: C, 63.85; H, 3.94; N, 12.88; S, 7.45.

*4.8. General procedure for synthesis of the quinoline derivatives **24a**,**b***

A mixture of cyanoacetamides **2a**,**b** (5 mmol) and salicylaldehyde (0.61 g, 5 mmol) was refluxed in EtOH (25 mL) in the presence of piperidine (7 drops) for 2 h. Then, the reaction mixture was allowed to cool to room temperature, the formed crude product was collected by filtration washed with EtOH and recrystallized from DMF to afford **24a,b** respectively.

*4.8.1. N-(3-cyano-4-phenylthiophen-2-yl)-2-oxo-1,2-dihydroquinoline-3-carboxamide (**24a**)*

yellow crystals, yield: 80%, m.p.: 258–259 °C; IR (KBr): νcm^{-1} 3436, 3327 (2NH), 2214 (CN), 1712, 1677 (2CO); ^{1}H NMR (DMSO-d_6): δ = 7.26 (s, 1H, thiophene H-5), 7.33–7.87 (m, 9H, Ar–H), 8.68 (s. 1H, quinoline H-4), 10.12 (s, 1H, NH) and 14.60 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 83.55, 105.08, 115.41 (CN), 116.43, 116.73, 125.42, 126.83, 127.18, 127.25, 127.74, 128.58, 128.79, 128.89, 129.85, 135.39, 138.66, 153.42, 154.08 and 166.30 ppm (Ar-C and CO); MS (EI): m/z (%) 371 (M$^+$, 43.56), 372 (M$^+$+1, 17.25). Anal. calcd. for $C_{21}H_{13}N_3O_2S$ (371.42): C, 67.91; H, 3.53; N, 11.31; S, 8.63. Found: C, 67.87; H, 3.59; N, 11.35; S, 8.57.

*4.8.2. Crystallographic analysis for **24a***

The crystals were mounted on a glass fiber. All measurements were performed on a Rigaku R-AXIS RAPID diffractometer using filtered Mo-Kα radiation. The data were collected at a temperature of 20 ± 1 °C to a maximum 2θ value of 55.0° using the ω scanning technique. The structure was solved by charge flipping method and expanded using Fourier techniques. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model.

4.8.3. Crystal data

$C_{21}H_{13}N_3O_2S$, M = 371.42, triclinic, a = 3.8641(3) Å, b = 12.0951(9) Å, c = 17.999(2) Å, V = 833.0(1) Å^3, α = 86.818(6)°, β = 86.151(6)°, γ = 83.561(6)°, space group: P-1, Z = 2, D_{calc} = 1.481 g cm^{-3}, No. of reflection measured 3701, $2\theta_{max}$ = 55.0°, $R1$ = 0.099. Fig. 6 illustrates the structure as determined. Full data can be obtained on request from the CCDC [28].

*4.8.4. N-[4-(4-Chlorophenyl)-3-cyanothiophen-2-yl]-2-oxo-1,2-dihydroquinoline-3-carboxamide(**24b**)*

yellow crystals, yield: 80%, m.p.: 265–266 °C; IR (KBr): νcm^{-1} 3423, 3329 (2NH), 2217 (CN), 1712, 1678 (2CO); ^{1}H NMR (DMSO-d_6): δ = 7.31 (s, 1H, thiophene H-5), 7.38–7.42 (m, 2H, Ar–H), 7.56 (d, J = 8.0 Hz, 2H, Ar–H), 7.66–7.72 (m, 3H, Ar–H), 7.93 (d, J = 9.2 Hz, 1H, Ar–H), 8.76 (s. 1H, quinoline H-4), 10.18 (s, 1H, NH) and 14.74 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 83.04, 105.54, 115.25 (CN), 115.69, 116.55, 124.68, 125.23, 128.37, 128.44, 128.63, 128.71, 129.77, 130.26, 133.87, 135.20, 136.07, 152.90, 153.24 and 166.27 ppm (Ar-C and CO); MS (EI): m/z (%) 405 (M$^+$, 52.66), 406 (M$^+$+1, 18.93). Anal. calcd. for $C_{21}H_{12}ClN_3O_2S$ (405.87): C, 62.15; H, 2.98; N, 10.35; S, 7.90. Found: C, 62.11; H, 2.92; N, 10.41; S, 7.88.

*4.9. General procedure for the preparation of pyridone **29a-c***

Independent solutions of cyanoacetamides **2a** (2.67 g, 10 mmol) and enaminones **4a**–**c** (10 mmol) in AcOH (20 mL) containing anhydrous sodium acetate (1.5 g) were stirred at reflux for 4 h. Then, the reaction mixtures were cooled to room temperature and poured onto ice cold water. The crude products were collected by filtration, washed with water and recrystallized from the appropriate solvent to afford the pyridine derivatives **29a**–**c**.

*4.9.1. N-(3-cyano-4-phenylthiophen-2-yl)-2-oxo-6-phenyl-1,2-dihydropyridine-3-carboxamide(**29a**)*

Recrystallized from an EtOH/DMF (1:1) mixture as orange crystals, yield: 70%, m.p.: 289–290 °C; IR (KBr): νcm^{-1} 3445, 3331 (2NH), 2214 (CN), 1674, 1655 (2CO); ^{1}H NMR (DMSO-d_6): δ = 6.97

(d, J = 7.8 Hz, 1H, H-5), 7.30 (s, 1H, thiophene H), 7.42 (t, J = 7.8 Hz, 1H, Ar–H), 7.50 (t, J = 7.8 Hz, 2H, Ar–H), 7.56–7.59 (m, 3H, Ar–H), 7.67 (d, J = 7.8 Hz, 2H, Ar–H), 7.87 (d, J = 7.8 Hz, 2H, Ar-–H), 8.53 (d, J = 7.8 Hz, 1H, H-4), 12.97 (s, 1H, NH) and 13.90 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6 at 100 °C): δ = 93.26, 106.59, 114.93, 115.88 (CN), 116.21, 127.72, 128.08, 128.67, 129.25, 129.46, 131.62, 132.39, 134.12, 138.07, 145.66, 151.68, 152.53, 161.69 and 163.94 ppm (Ar-C and CO); MS (EI): m/z (%) 397 (M^+, 22.10), 398 (M^++1, 6.98). Anal. calcd. for $C_{23}H_{15}N_3O_2S$ (397.46): C, 69.51; H, 3.80; N, 10.57; S, 8.07. Found: C, 69.48; H, 3.85; N, 10.58; S, 8.13.

4.9.2. N-(3-cyano-4-phenylthiophen-2-yl)-2-oxo-6-(thiophen-2yl)-1,2-dihydropyridine-3-carboxamide(**29b**)

Recrystallized from an EtOH/DMF (1:1) mixture as orange crystals, yield: 68%, m.p.: 223–225 °C; IR (KBr): νcm^{-1} 3421, 3265 (2NH), 2215 (CN), 1674, 1659 (2CO); ^{1}H NMR (DMSO-d_6): δ = 6.92 (d, J = 8.4 Hz, 1H, H-5), 7.29 (t, J = 4.8 Hz, 1H, thiophene H), 7.33 (s, 1H, thiophene H), 7.50–7.64 (m, 5H, Ar–H), 7.94 (d, J = 4.8 Hz, 1H, thiophene H), 8.09 (d, J = 4.8 Hz, 1H, thiophene H), 8.56 (d, J = 8.4 Hz, 1H, H-4), 13.24 (s, 1H, NH) and 13.92 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6 at 100 °C): δ = 92.97, 105.06, 115.50 (CN), 116.02, 127.20, 128.38, 128.87, 128.92, 129.21, 129.64, 131.05, 133.31, 134.04, 137.41, 145.32, 150.34, 159.24, 160.33 and 161.47 ppm (Ar-C and CO); MS (EI): m/z (%) 403 (M^+, 50.08), 404 (M^++1, 24.50). Anal. calcd. for $C_{21}H_{13}N_3O_2S_2$ (403.48): C, 62.51; H, 3.25; N, 10.41; S, 15.89. Found: C, 62.43; H, 3.32; N, 10.34; S, 15.98.

4.9.3. N-(3-cyano-4-phenylthiophen-2-yl)-6-(6-methylpyridin-2yl)-2-oxo-1,2-dihydropyridine-3-carbox- amide(**29c**)

Recrystallized from an EtOH/DMF (1:1) mixture as green crystals, yield: 63%, m.p.: 274–275 °C; IR (KBr): νcm^{-1} 3429, 3287 (2NH), 2214 (CN), 1677, 1663 (2CO); ^{1}H NMR (DMSO-d_6): δ = 2.61 (s, 3H, CH_3), 7.26 (s, 1H, thiophene H), 7.37 (d, J = 7.2 Hz, 1H, H-5), 7.40–7.44 (m, 2H, Ar–H), 7.48 (t, J = 7.8 Hz, 2H, Ar–H), 7.64 (d, J = 7.8 Hz, 2H, Ar–H), 7.89 (t, J = 7.8 Hz, 1H, Ar–H), 8.01 (d, J = 7.8 Hz, 1H, Ar–H), 8.58 (d, J = 7.2 Hz, 1H, H-4), 12.46 (s, 1H, NH) and 13.91 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6 at 90 °C): δ = 23.38 (CH_3), 92.66, 104.90, 113.99, 115.15 (CN), 117.41, 118.83, 125.18, 126.86, 127.85, 128.40, 133.22, 137.31, 137.75, 145.22, 145.99, 147.53, 150.59, 158.34, 160.56 and 161.96 ppm (Ar-C and CO); MS (EI): m/z (%) 412 (M^+, 27.85), 413 (M^++1, 8.25). Anal. calcd. for $C_{23}H_{16}N_4O_2S$ (412.47): C, 66.98; H, 3.91; N, 13.58; S, 7.77. Found: C, 66.96; H, 3.87; N, 13.64; S, 7.69.

4.10. General procedure for the preparation of **30a**,**b**

A cold solution of benzenediazonium chloride (10 mmol) was prepared by adding a solution of sodium nitrite (1.4 g dissolved in 10 mL water) to cold solution of aniline hydrochloride (0.93 g of aniline in 10 mL, 6M HCl) with stirring. The resulting solution of benzenediazonium chloride was then added to a cold solution of cyanoacetamide **2a** (2.67 g, 10 mmol) in pyridine or to a solution of the cyanoacetamide **2c** (2.77 g, 10 mmol) in ethanol (50 mL) in the presence of sodium acetate trihydrate (4.2 g, 30 mmol). The reaction mixture was stirred at room temperature for 1 h. In case of **30a** the mixture was poured into ice cold water and neutralized with HCl, the formed solid product was collected by filtration and washed with water then EtOH. While in case of **30b** the product was collected directly from the reaction mixture by filtration and washed with water then EtOH. Finally the obtained products were recrystallized from an EtOH/DMF (1:2) mixture to afford **30a**,**b** respectively.

4.10.1. (E)-2-cyano-N-(3-cyano-4-phenylthiophen-2-yl)-2-(phenylhydrazono)acetamide (**30a**)

reddish browncrystals, yield: 65%, m.p.: 218–220 °C; IR (KBr): νcm^{-1} 3377, 3227 (2NH), 2222, 2207 (2CN), 1685 (CO); ^{1}H NMR (DMSO-d_6): δ = 7.19 (t, J = 7.6 Hz, 1H, Ar–H), 7.41 (s, 1H, thiophene H-5), 7.42–7.45 (m, 3H, Ar–H), 7.51 (t, J = 7.6 Hz, 2H, Ar–H), 7.63 (d, J = 7.6 Hz, 2H, Ar–H), 7.70 (d, J = 7.6 Hz, 2H, Ar–H), 10.98 (s, 1H, NH) and 12.44 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 95.96, 105.10, 110.80, 114.68, 116.45 (CN), 116.53 (CN), 125.08, 127.29, 128.36, 128.91, 129.26, 133.36, 137.95, 141.74, 150.24 and 159.47 ppm (Ar-C and CO); MS (EI): m/z (%) 371 (M^+, 43.98), 372 (M^++1, 10.70). Anal. calcd. for $C_{20}H_{13}N_5OS$ (371.42): C, 64.68; H, 3.53; N, 18.86; S, 8.63. Found: C, 64.74; H, 3.55; N, 18.90; S, 8.69.

4.10.2. (E)-4-cyano-5-[2-cyano-2-(phenylhydrazono)acetylamino]-3-methylthiophene-2-carboxylic acid ethyl ester (**30b**)

yellow crystals, yield: 73%, m.p.: 249–250 °C; IR (KBr): νcm^{-1} 3367, 3227 (2NH), 2215 (br, 2CN), 1688 (CO); ^{1}H NMR (DMSO-d_6): δ = 1.29 (t, 3H, J = 7.2 Hz, CH_3CH_2), 2.51 (s, 3H, CH_3), 4.26 (q, 2H, J = 7.2 Hz, CH_3CH_2), 7.20 (t, J = 7.6 Hz, 1H, Ar–H), 7.42 (t, J = 7.6 Hz, 2H, Ar–H), 7.66 (d, J = 7.6 Hz, 2H, Ar–H), 11.22 (s, 1H, NH) and 12.53 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 14.09 (CH_3), 14.32 (CH_3), 60.91 (CH_2), 98.83, 104.67, 110.60, 113.30, 116.59, 117.72, 125.32, 129.29, 141.66, 143.45, 150.80, 159.76 and 161.32 ppm (2 CN, Ar-C and CO); MS (EI): m/z (%) 381 (M^+, 27.75), 382 (M^++1, 7.90). Anal. calcd. for $C_{18}H_{15}N_5O_3S$ (381.42): C, 56.68; H, 3.96; N, 18.36; S, 8.41. Found: C, 56.71; H, 3.92; N, 18.41; S, 8.35.

4.10.3. Crystallographic analysis for **30b**

The crystals were mounted on a glass fiber. All measurements were performed on Bruker X8 Prospector. The data were collected at a temperature of 20 ± 1 °C to a maximum θ value of 66.61° using the ω scanning technique. The structure was solved by direct method using SHELXS-97 (Sheldrick, 2008) and refined by Full-matrix least-squares on F^2. The non-hydrogen atoms were refined anisotropically. Data were corrected for absorption effects using the multi-scan method (SADABS).

4.10.4. Crystal data

$C_{18}H_{15}N_5O_3S$, M = 381.42, monoclinic, a = 8.0336(4) Å, b = 14.2787(7) Å, c = 20.2833(10) Å, V = 2312.6(2) Å^3, $\alpha = \gamma = 90.00°$, β = 96.305(3)°, space group: P 1 21/n 1, Z = 4, D_{calc} = 1.305 g cm^{-3}, No. of reflection measured 3987, θ_{max} = 66.61°, $R1$ = 0.06. Fig. 7 illustrates the structure as determined. Full data can be obtained on request from the CCDC [31].

4.11. 4-Cyano-5-[2-cyano-2-(cyanomethylphenylhydrazono) acetylamino]-3-methylthiophene-2-carbo- xylic acid ethyl ester (**31**)

To a solution of **30b** (1.9 g, 5 mmol) in a mixture of DMF (2 ml) and triethylamine (15 ml) was added the chloroacetonitrile (0.45g, 6 mmol) with external cooling. The reaction mixture was refluxed for 4 h and left to cool to room temperature. The obtained residual product was triturated with ethanol to give a solid product that was collected by filtration, washed with water and recrystallized from ethanol giving yellow, yield: 78%, m.p.: 188–189 °C; IR (KBr): νcm^{-1} 3446 (NH), 2208 (br, 3CN), 1678 (CO); ^{1}H NMR (DMSO-d_6): δ = 1.28 (t, 3H, J = 7.2 Hz, CH_3CH_2), 2.51 (s, 3H, CH_3), 4.21 (q, 2H, J = 7.2 Hz, CH_3CH_2), 4.85 (s, 2H, CH_2), 7.07 (t, J = 7.6 Hz, 1H, Ar–H), 7.37 (t, J = 7.6 Hz, 2H, Ar–H), 7.45 (d, J = 7.6 Hz, 2H, Ar–H) and 15.32 ppm (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 14.33 (CH_3), 14.56 (CH_3), 45.57(CH_2), 59.88 (CH_2), 98.13, 112.23, 113.86, 114.25, 114.37, 117.16, 118.15, 123.50, 129.55, 142.28, 143.28, 162.33, 164.32 and 164.90 ppm (2 CN, Ar-C and CO); MS (EI): m/z (%) 420 (M^+, 25.75), 421 (M^++1, 6.85). Anal. calcd. for $C_{20}H_{16}N_6O_3S$ (420.45): C, 57.13; H, 3.84; N, 19.99; S, 7.63. Found: C, 57.15; H, 3.79; N, 20.03; S, 7.69.

5. Conclusions

In this investigation the cyanoacetamides, of the multi-substituted 2-aminothiophenes were used as key synthons for the preparation of wide variety of a novel, pyrido[1,2-*a*]thieno[3,2-*e*] pyrimidine derivatives. In addition it was also used for the synthesis of a new class of quinoline and pyridine derivatives. The objective of this study was verified by synthesizing the above class of compounds and investigating the antimicrobial activities for these new compounds with the hope of discovering new structure leads serving as potent antibacterial and antifungal agents, the results of biological evaluations demonstrate that members from these compounds have promising antimicrobial activities against Gram negative bacteria, Gram positive bacteria and Yeast.

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