



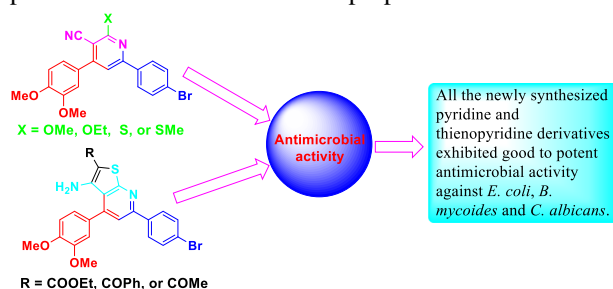
Novel biologically active pyridine derivatives: Synthesis, structure characterization, in vitro antimicrobial evaluation and structure-activity relationship

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Abstract

The rate of microbial resistance has continued to rise significantly as the availability of new antibiotics has declined. A new series of pyridine and thienopyridine derivatives were designed, synthesized and tested as antimicrobial agents. The reaction of 4-bromo acetophenone and veratraldehyde (3,4-dimethoxy benzaldehyde) in ethanol and sodium hydroxide solution afforded the corresponding chalcone which was used as a suitable precursor to prepare a new series of pyridine derivatives. The treatment of the latter chalcone with 2-cyanothioacetamide afforded the corresponding pyridinethione which was used as a precursor to synthesize the targeted thienopyridine derivatives in good to excellent yield by the reaction with 2-chloro-*N*-arylacetamide derivatives, α -haloketones, methyl iodide or chloroacetonitrile in one or two steps. The structure of the synthesized compounds was confirmed chemically by their preparations with other pathways and their spectral data. The newly synthesized pyridine and thienopyridine derivatives exhibited good to strong antimicrobial activity against microbial strains *E. coli*, *B. mycoides* and *C. albicans*. With maximal antimicrobial activity against *B. mycoides* (33 mm) and *C. albicans* (29 mm), respectively, compounds **12a** and **15** demonstrated the highest inhibition zone. Compound **12a** prevented the growth of *E. coli*, at MIC level of 0.0195 mg/mL, and *B. mycoides* and *C. albicans* at MIC level below than 0.0048 mg/mL, respectively. Additionally, compound **15** prevented the visible growth of *E. coli*, *B. mycoides*, and *C. albicans* at MIC values of >0.0048, 0.0098, and 0.039 mg/mL, respectively. The relation between the chemical structure of the synthesized pyridine and thienopyridine compounds and their antimicrobial properties was discussed in the SAR study.



Keywords Pyridine · Thienopyridine · Chalcone · Veratraldehyde · Antimicrobial

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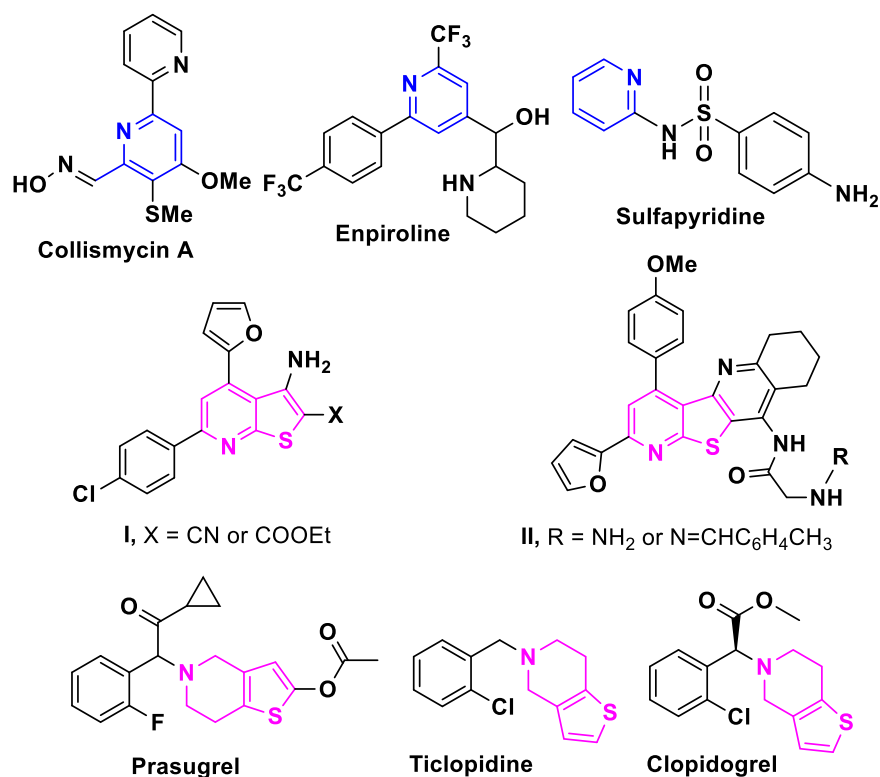
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Introduction

Fused heterocyclic compounds containing pyridine moiety represent a variety of biological activities in different areas [1]. Pyridine scaffold is an essential core in the chemical structure of a variety of approved drugs in the pharmaceutical market (Fig. 1) [1–6]. Numerous pyridine derivatives have been studied for their biological and pharmacological activities; some of these compounds have been shown to

Fig. 1 Pyridine and thienopyridine scaffold-bearing compounds in therapeutic applications



have antibacterial properties such as Sulfapyridine (Fig. 1). On the other hand, one of the most common pyridine derivatives is known as thienopyridine compounds. These scaffolds are employed as precursors in the synthesis of target agrochemicals and pharmaceuticals (Fig. 1). Fused thienopyridine derivatives have been reported associated with different medicinal and biological properties including anti-inflammatory [7], antibacterial [8], antifungal [9], anticancer [10], and antimicrobial agents [11]. Ratab et al. [12]. reported a new series thienopyridine derivatives with potent antifungal activity (compounds I, Fig. 1), likewise, Mohi El-Deen et al. [13]. reported a novel series of thienopyridine derivatives with high antibacterial properties (compounds II, Fig. 1). Moreover, some drugs in the pharmaceutical market contain thienopyridine moiety in their structures such as Clopidogrel [14], Ticlopidine [15], and Prasugrel [16] (Fig. 1).

Antibiotics were arguably the most successful chemotherapeutic agents developed in the 20th century. After many decades from their discovery and introduction to the market, antibiotics have saved countless lives [17]. Modern medicine depends on antibiotics' capacity to both cure and prevent a wide range of illnesses. It is well known that bacteria may become resistant to antibiotics [18]. Antibiotics were often taken for granted since, in the beginning, there were enough new ones available to battle the resistance that was being shown [19]. On the other hand, the level of bacterial resistance has continued to rise

dramatically as the availability of new antibiotics has decreased. Overuse of antibiotics combined with the ongoing spread and development of mobile genetic resistance elements over the past 20 years has led to an increase in the prevalence of multidrug resistance [17]. Contrasting sharply with the current level of investment in antimicrobial development is the growing need for new drugs to treat potentially fatal infections brought on by the global spread of multidrug-resistant bacterial pathogens. This is especially true for small molecules derived from natural products and synthetic compounds. To combat the threat that antimicrobial resistance poses to public health, novel agents with inventive chemistry and modes of action are sorely needed on a global scale [20].

Therefore, due to these facts and in continuation of our previous work on the synthesis of new organic compounds with evaluation of their biological activities [21–30]. We report herein reliable synthetic approaches for a new series of pyridine and thienopyridine derivatives and evaluation of their biological activities as antimicrobial agents.

Results and discussion

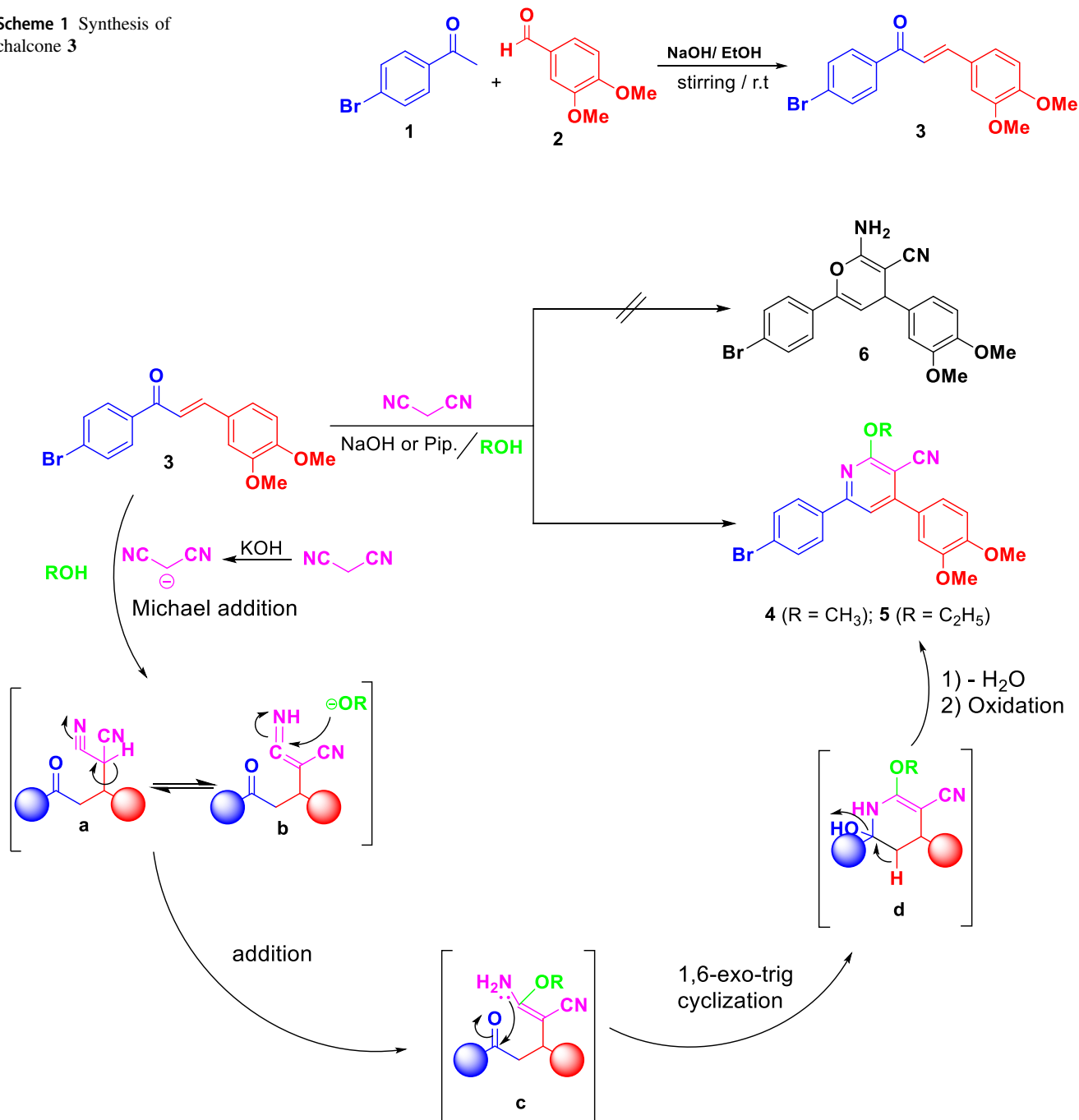
Chemistry

According to the literature [31, 32], 4-bromo acetophenone (1) and vanillin (3,4-dimethoxy benzaldehyde) (2)

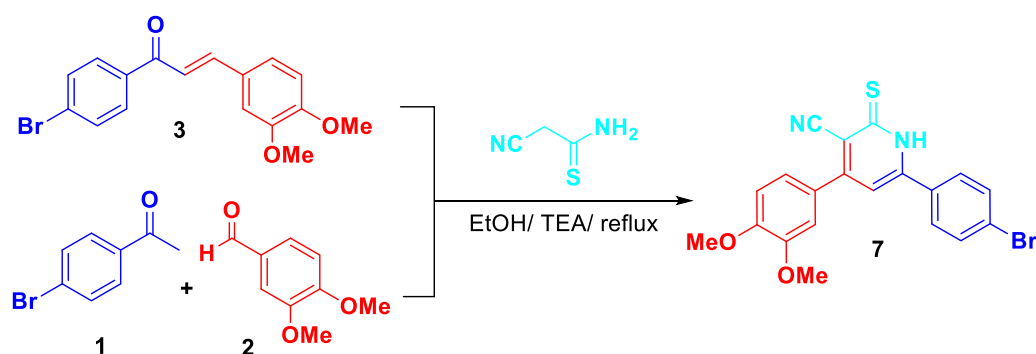
were reacted to produce 3-(3,4-dimethoxyphenyl)-1-(4-bromophenyl)prop-2-en-1-one (**3**) in the presence of piperidine or NaOH. Compound **3** reacted with malononitrile in methanol or ethanol as a suitable solvent to yield the unexpected alkoxy-pyridine compounds **4** and **5**, respectively (rather than 4-H-cyanopyran **6**) (Scheme 1). The lone pairs of the hydroxy groups in the methanol or ethanol solvent, as shown in Scheme 2, are essential to the suggested reaction mechanism and the end products **4** and **5**

(Scheme 2) [33]. The chemical structures of the synthesized compounds **4** and **5** were elucidated by spectral data and elemental analysis. The ^1H NMR spectrum of the methoxy-pyridine derivative **4** was characterized by the presence of a singlet signal at δ 4.21 (ppm) for the OCH_3 group, while the ^1H NMR spectrum of compound **5** showed a triplet signal (CH_3 group) at δ 1.45 (ppm) ($J = 7.2$ Hz) and a quartet signal (CH_2 group) at δ 4.63 (ppm) ($J = 7.2$ Hz) due to the ethyl group. The mass spectrometry of compounds **4**

Scheme 1 Synthesis of chalcone **3**



Scheme 2 Synthesis of compounds **4** and **5** with the expected reaction mechanism (in case of using KOH as a catalyst)



Scheme 3 Synthesis of 6-(4-bromophenyl)-4-(3,4-dimethoxyphenyl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile (7)

and **5** showed two peaks at $m/z = 425$ and $m/z = 438$ for both **4** and **5**, respectively.

The intermediate (a) was produced by a Michael addition reaction between compound **3** and malononitrile. The expected intermediate (c) was then produced by the nucleophilic attack of the alkoxide anion (RO^-) from the solvent (methanol in case of (**4**) or ethanol in case of (**5**)) over the ketone-imine (b). The expected intermediate (d) was then formed by intramolecular *N*-cyclization of the intermediate (c), followed by the elimination of the water molecule, and then oxidative aromatization to afford the corresponding products **4** or **5**. It is important to note that, the expected reaction mechanism in Scheme 2 is could be considered only by using KOH as a catalyst.

Treatment of compound **3** with 2-cyanothioacetamide in ethanol in the presence of trimethylamine afforded orange precipitate, of the corresponding cynopyridinethione derivative (**7**) with excellent yield (94%, Scheme 3). In the same context, cynopyridinethione (**7**) could be obtained as one pot step *via* the reaction of 4-bromoacetophenone (**1**), 3,4-dimethoxybenzaldehyde (**2**) and 2-cyanothioacetamide in a molar ratio of 1:1:1 in ethanol under reflux for 4 hours as shown in Scheme 3. $^1\text{H-NMR}$ spectrum of pyridinethione (**7**), showed a multiplet signal at chemical shift δ 3.82–3.98 (ppm) assigned to the protons of the two methoxy groups, while the aromatic protons were observed in the aromatic zone at δ 6.88–7.16 and 7.26–7.73 (ppm). The CN group is observed with a band at 2226 cm^{-1} in the infrared spectrum, and the molecular ion peak of **7** is identified by a peak (M^+) at m/z (EI) = 426 in the mass spectrum.

The cynopyridinethione (**7**) and 2-chloro-*N*-arylaceta-mide derivatives (**8a–c**) were refluxed in ethanol and with a few drops of Et_3N to yield the corresponding compounds **9a–c**, respectively. The CH_2 group in $^1\text{H-NMR}$ spectra of each compound was reported as a singlet signal at δ 4.33, 4.32, and 4.31 (ppm), respectively. It is noteworthy to mention that, the appearance of these signals (of the CH_2 groups) confirms the open structure form as visualized in Scheme 4. As a result, compounds **9a–c** were cyclized by

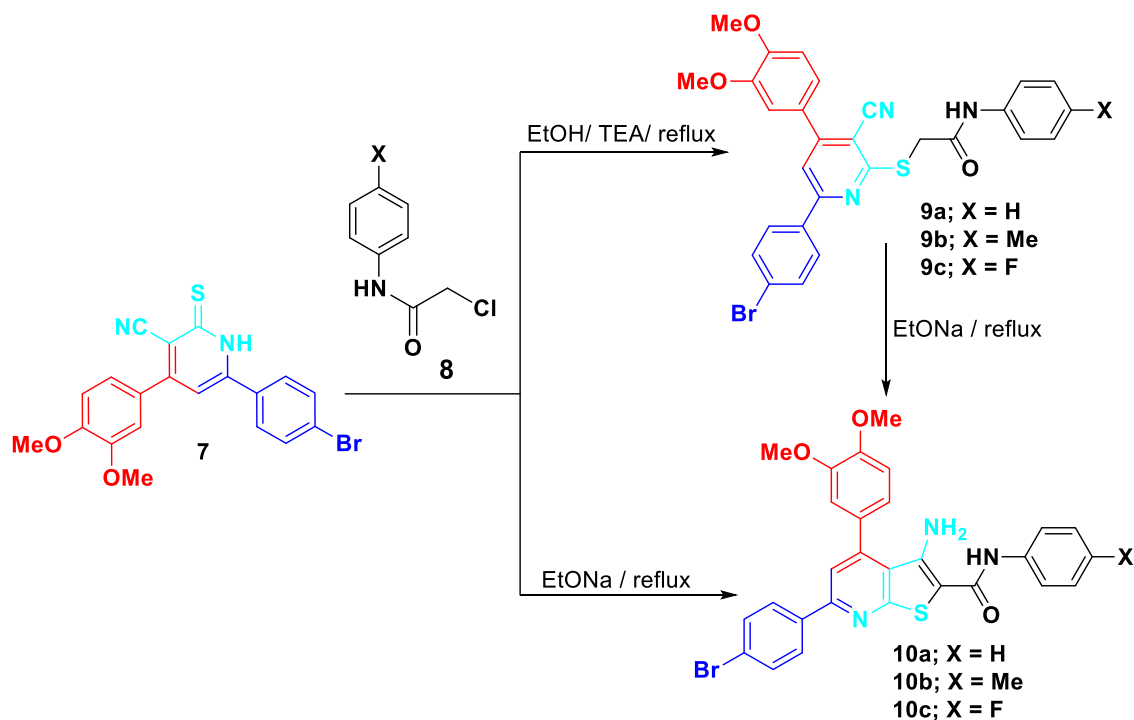
refluxing in sodium-ethoxide solution to yield the corresponding thienopyridine derivatives (**10a–c**). Compounds **10a–c**, on the other hand, were obtained in a single step by treating **7** with **8a–c** in a refluxing sodium-ethoxide solution (Scheme 4). Elemental analysis and spectral data were used to prove the chemical structure of compounds **10a–c** (Scheme 4). In this regard, the $^1\text{H-NMR}$ spectra of compounds **10a–c** confirmed that the singlet signals of the CH_2 group were missed and new signals for the NH_2 groups appeared at 6.01, 5.91, and 5.90 (ppm), respectively (see experimental part).

Moreover, the reaction of **7** with some α -halo ketone derivatives such as chloroacetone, phenacyl chloride and ethyl chloroacetate gave the corresponding *S*-alkyl derivatives (**11a–c**), respectively (Scheme 5). Cyclization of **11a–c** was carried out by refluxing these compounds in freshly prepared sodium ethoxide solution to give the corresponding thieno[2,3-*b*]pyridine derivatives (**12a–c**), respectively. The chemical structure of both *S*-alkyl derivatives (**11a–c**) and thieno[2,3-*b*]pyridine derivatives (**12a–c**) was confirmed by the correct elemental analyses and spectral data. On the other hand, the thieno[2,3-*b*]pyridine derivatives (**12a–c**) could be prepared directly as a one-pot reaction by the treatment of compound **7** with α -halo carbonyl compounds in the presence of sodium ethoxide (Scheme 5). Similarly, compound **7** was reacted with methyl iodide to give the corresponding *S*-methyl derivative **13** (Scheme 5). The CN group in the IR spectrum of **13** showed a band at 2211 cm^{-1} . Also, the $^1\text{H-NMR}$ spectrum of **13** revealed the methyl group at δ 2.72 (ppm) as a singlet, while the methoxy groups were assigned at δ 3.81 and 3.83 (ppm). In the mass spectrometry, the highest recorded peak of **13** showed at m/z (EI) = 441 (56%) in mass spectrometry.

Furthermore, compound **7** was reacted with chloroacetonitrile to give the *S*-alkyl derivative (**14**) which was cyclized in refluxed sodium ethoxide solution to afford compound **15** as shown in Scheme 6. The formation of compound **15** rather than **16** (Scheme 6) could be attributed

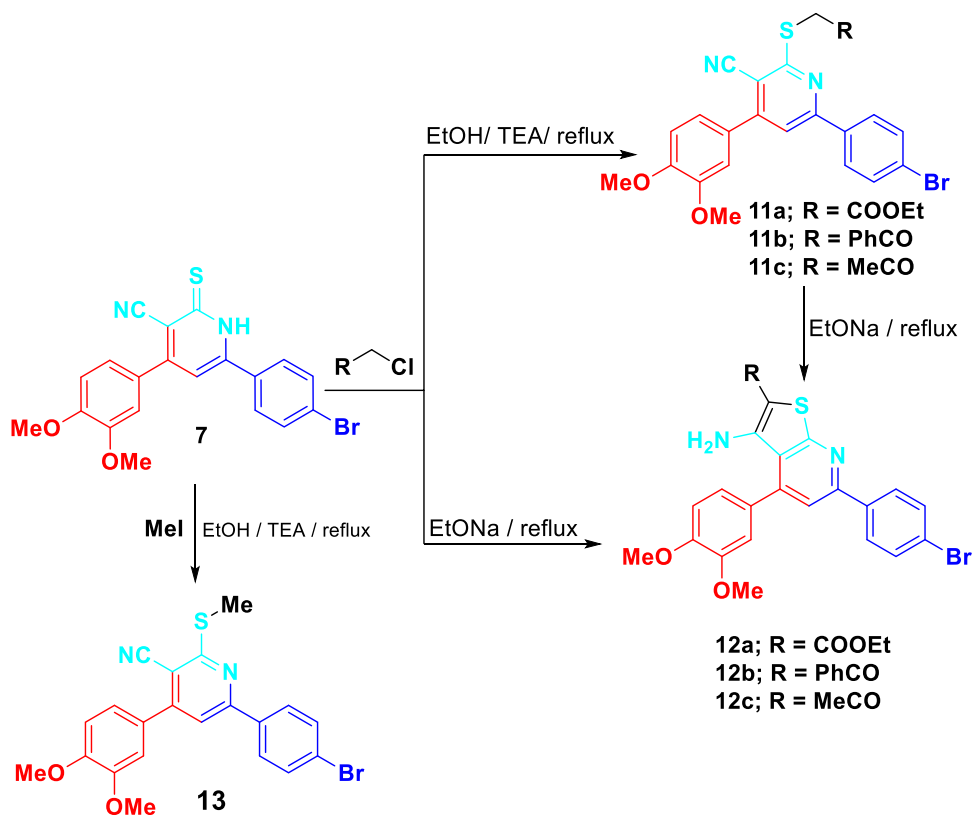
to the hydrolysis of the CN group to the amide group (Scheme 5). The IR spectrum of compound **15** showed a

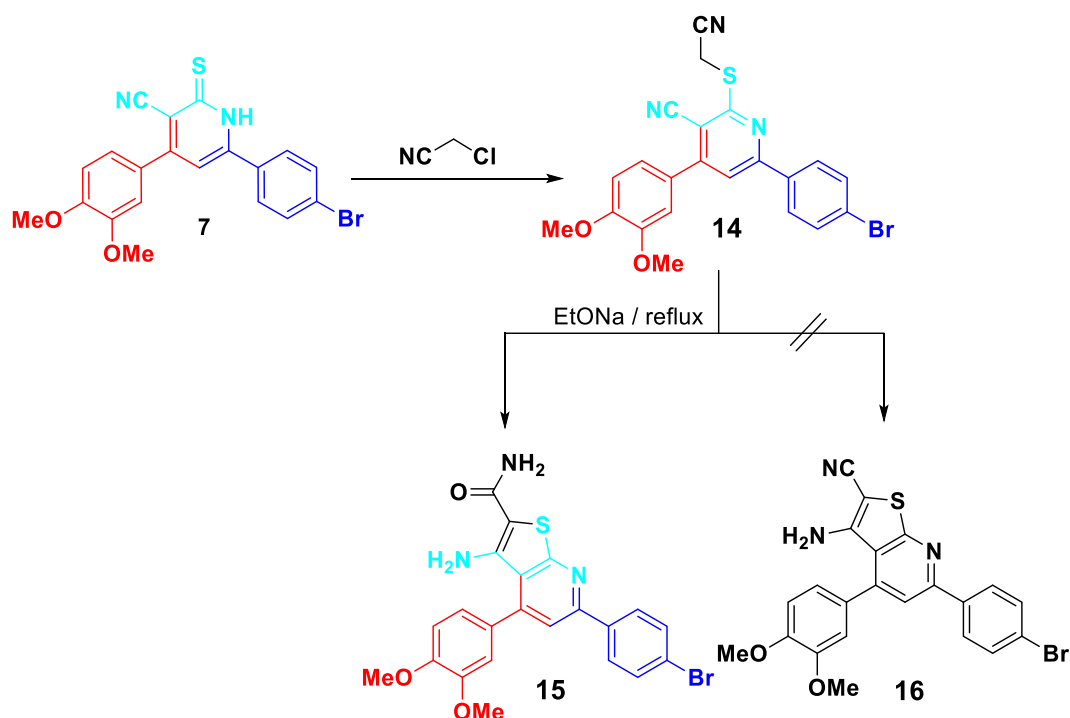
disappearance of the cyano group and a new absorption band at 1641 cm^{-1} for the C=O group. Moreover, in the



Scheme 4 Synthetic pathway for the preparation of compounds **9a-c** and **10a-c**

Scheme 5 Synthesis of compounds **11a-c**, **12a-c** and **13**





Scheme 6 Synthetic pathway for the newly synthesized compounds **14** and **15**

^1H NMR spectrum, two singlet signals at δ 5.89 and 7.18 (ppm) could be attributed to the presence of two NH_2 groups. The ^{13}C NMR spectrum of compound **15** showed a signal at δ 166.9 (ppm) owing to the presence of the $\text{C}=\text{O}$ group. The mass spectrometry (EI) showed a peak at $m/z = 483$ corresponding to the molecular ion of **15**.

Antimicrobial activity

To test the antibacterial potency of the produced compounds, *E. coli*, *B. mycoides*, and *C. albicans* were used as representations for Gram-negative, Gram-positive bacterial strains, and a non-filamentous fungal strain, respectively. These three typical microorganisms were used in the well diffusion experiment for the produced compounds. In general, the compositions and functional groups included in each synthetic chemical have a significant impact on the resistance of microbial strains to such compounds [34–36].

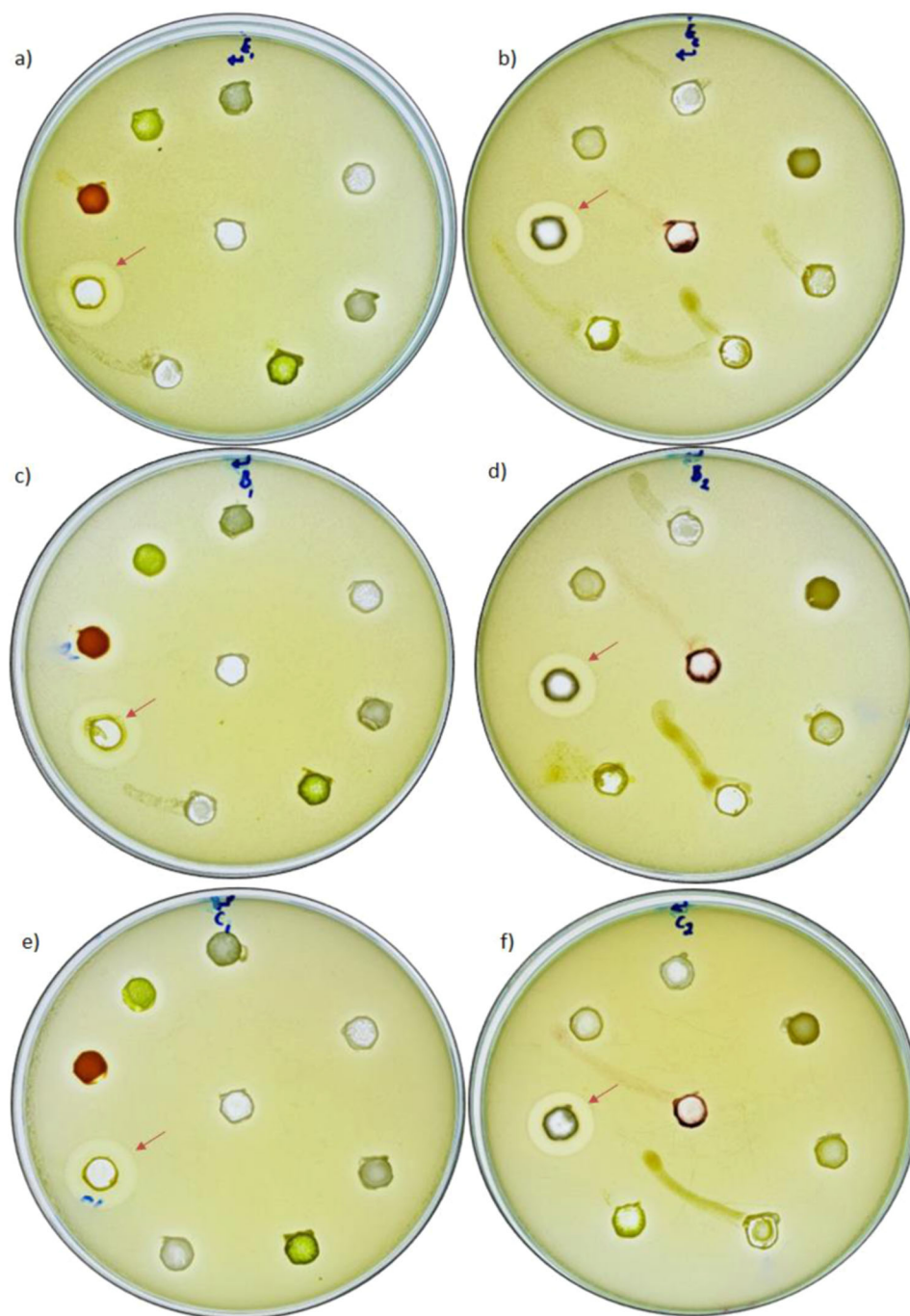
The results, which show how various tested microorganisms react differently to the synthesized compounds **4–15**, are listed in Table 1 and shown in Fig. 2. Compounds **12a** and **15** showed the greatest inhibition zone against the tested microbial strains, with maximum antibacterial activity against *B. mycoides* (33 mm) and *C. albicans* (29 mm), respectively, which are higher than the activity exerted by the applied control antibiotics (Ampicillin 10 μg , Gentamicin 10 μg , and Tobramycin 10 μg). Contrarily, *C. albicans* showed the least reaction (inhibition zone of 16 mm) to

Table 1 Assessment of antimicrobial properties of compounds **4–15** using the agar diffusion technique

Compound	Inhibition zone (mm)		
	<i>E. coli</i>	<i>B. mycoides</i>	<i>C. albicans</i>
4	20	23	20
5	19	20	18
7	20	25	21
9a	18	19	18
9c	23	20	16
10a	21	18	16
10b	20	17	17
10c	22	22	18
11b	21	23	18
11c	25	21	17
12a	32	33	31
12b	18	17	18
12c	21	25	20
13	21	18	19
14	20	19	18
15	28	28	29
Ampicillin 10 μg	20	12	14
Gentamicin 10 μg	12	11	12
Tobramycin 10 μg	13	10	12

compounds **9a**, **9c** and **10a** among the studied microbial strains (Table 1). However, the majority of the investigated

Fig. 2 Assessment of synthetic chemicals' antimicrobial properties using the agar diffusion technique. Arrows indicate the selected compounds **12a** (a, c, e) and **15** (b, d, f) with the highest microbial inhibition activity against *E. coli* (a, b), *B. mycoides* (c, d), and *C. albicans* (e, f)

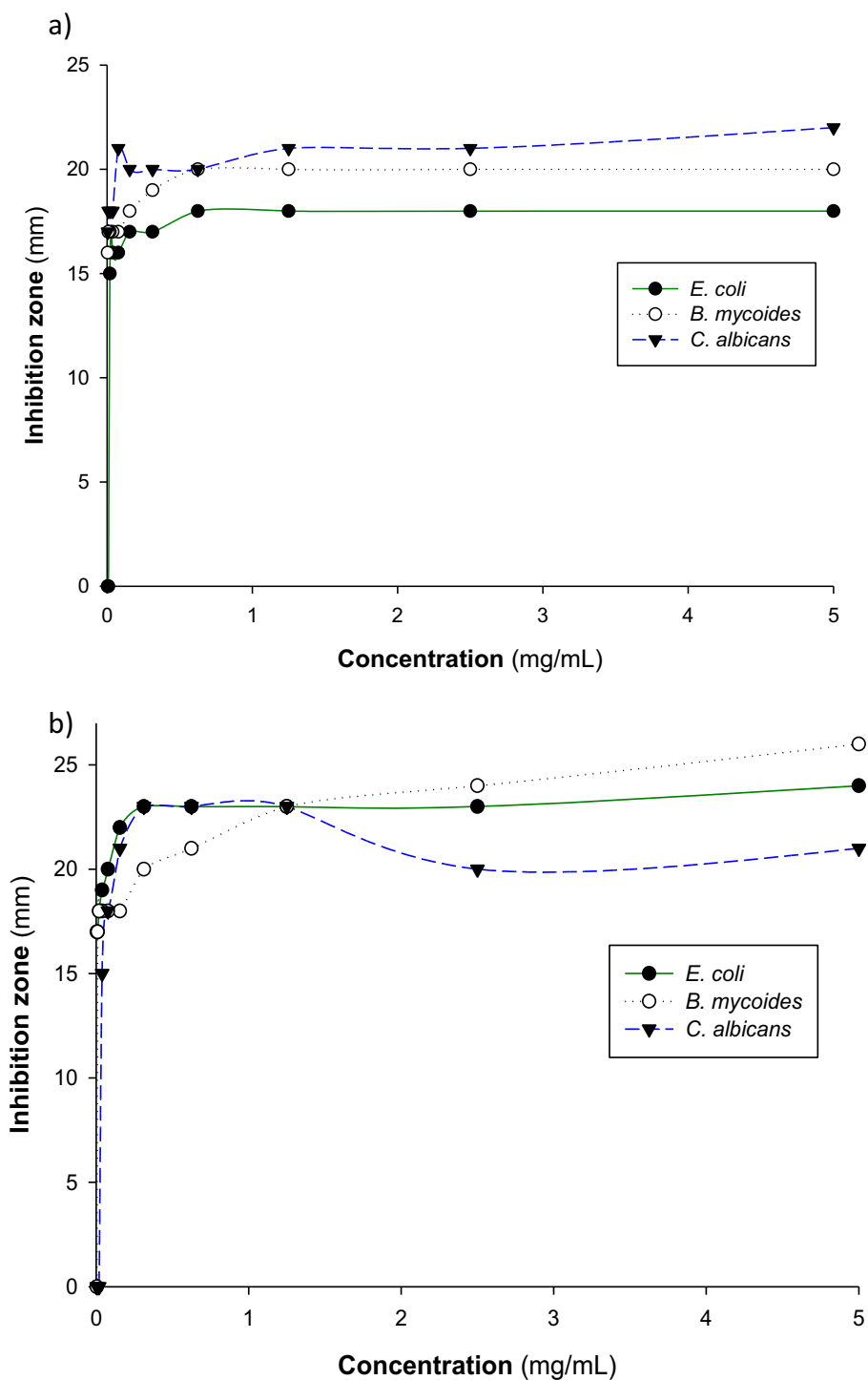


compounds exhibited modest antibacterial efficacy against the described microbes. Therefore, the highest two compounds **12a** and **15** were chosen to calculate their MIC values for the three investigated microbes.

Different concentrations of each compound were examined for their antibacterial activity during the 18-h incubation period to assess the antimicrobial activity durability of both **12a** and **15** compounds against the indicated pathogens. Figures 3, S1, S2, and Table S1 (supplementary data) show the results obtained and demonstrate that the

synthesized compounds have the ability to inhibit the tested microbes even at low concentrations. This behavior was also demonstrated by the measured MIC values (Table 2 and Fig. 3), which demonstrate that compound **12a** has lower MIC values than compound **15** (which has a greater capacity to inhibit microbial growth), with the exception of *E. coli*, which was inhibited by a lower concentration of compound **15**. *B. mycoides* and *C. albicans*' growth was suppressed by compound **12a** at MIC levels lower than 0.0048 mg/mL, whereas compound **15** was successful in

Fig. 3 Compounds **12a** (a) and **15** (b) antimicrobial activity at various doses



preventing their visible development at MIC levels of 0.0098 and 0.039 mg/mL, respectively (Table 2).

Structure-activity relationship (SAR)

Structure-activity relationship will be discussed to correlate the relation between the chemical structure of the synthesized pyridine and thienopyridine compounds and their

antimicrobial properties as visualized in Fig. 4. In general, most of the newly synthesized pyridine and thienopyridine derivatives (4–15) exhibited well to strong antimicrobial activity against pathogens *E. coli*, *B. mycoides* and *C. albicans*. Thienopyridine derivatives (10, 12 and 15) showed strong antimicrobial activity with the highest inhibition zone than that observed in the case of pyridine derivatives (4, 5, 7, 9, 11, 13 and 14). Compound 12a

(R = COOEt) prevented the growth of *E. coli*, at MIC level of 0.0195 mg/mL, and *B. mycooides* and *C. albicans* at MIC level below than 0.0048 mg/mL, respectively, while compound **15** (R = CONH₂) prevented the visible growth of *E. coli*, *B. mycooides*, and *C. albicans* at MIC values of >0.0048, 0.0098, and 0.039 mg/mL, respectively.

Conclusions

In summary, an efficient and convenient route to the synthesis of a new series of substituted pyridines. The newly synthesized pyridine derivatives were used for the preparation of a novel series of fused thienopyridine derivatives. All the newly synthesized pyridine and thienopyridine derivatives were tested for their antimicrobial activity against *E. coli*, *B. mycooides*, and *C. albicans* as representatives for Gram-negative, Gram-positive bacterial strains, and a non-filamentous fungal strain, respectively. The findings of the study indicated that the selected synthesized derivatives with the highest antimicrobial activity can be implemented in future studies to synthesize new antimicrobial agents.

Table 2 Minimum Inhibitory Concentration (MIC) values of compounds **12a** and **15** against represented microorganisms

Compound	MIC value (mg/mL)		
	<i>E. coli</i>	<i>B. mycooides</i>	<i>C. albicans</i>
12a	0.0195	>0.0048	>0.0048
15	> 0.0048	0.0098	0.039

Each sample (100 μ L) having the required concentration was placed into a 15 mm agar well, and culture plates were then incubated overnight (18 h) at 37 $^{\circ}$ C.

Materials and methods

General

All melting points were measured on a Gallenkamp melting point apparatus (Weiss Gallenkamp, London, UK). The infrared spectra were recorded in potassium bromide disks on Pye Unicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometers (Pye Unicam Ltd. Cambridge, England and Shimadzu, Tokyo, Japan, respectively). The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer (Varian, Palo Alto, CA, USA). ¹H NMR spectra were run at 300 MHz or (Joel, Japan) 500 MHz and ¹³C NMR spectra were run at 75.46 MHz or 100.06 MHz in deuterated chloroform (CDCl₃) or dimethyl sulfoxide (DMSO-*d*₆). Chemical shifts are given in parts per million and are related to that of the solvent. Mass spectra were recorded with a Shimadzu GCMS-QP-1000 EX mass spectrometer in EI (70 eV) model. Elemental analyses were carried out at the Micro analytical Centre of Cairo University, Giza, Egypt and recorded on Elementar-Vario EL (Germany) automatic analyzer. 3-(4-Bromophenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one (**3**) was synthesized by Claisen-Schmidt condensation according to literature [31, 32]. 4-bromo acetophenone and veratraldehyde (3,4-dimethoxy benzaldehyde) in a 1:1 molar ratio were dissolved in ethanol (10 mL). Aqueous NaOH solution (10%, 20 mL) was added to the reaction mixture. The reaction mixture was monitored by TLC using chloroform: methanol (4.8:0.2) as a solvent system. When at least one of the starting compounds finished, reactions were stopped. The reaction content was poured on ice water and neutralized by HCl (10%). The precipitate obtained was washed with cold water and ethanol, filtered, and dried. Chalcone **3** was used

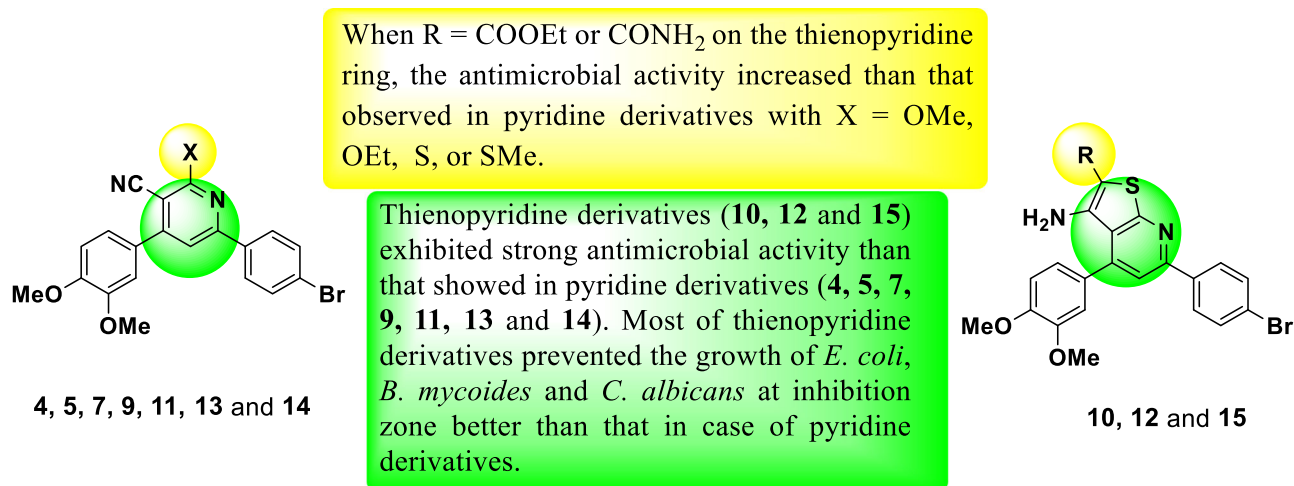


Fig. 4 Structure-activity relationship study of the synthesized pyridine and thienopyridine (**4–15**) compounds

for the synthesis of pyridine and thienopyridine derivatives without further purification [37].

Synthesis of compounds 4 and 5

Method A Sodium hydroxide (20%) was added to a mixture of malononitrile (10 mmol) and 3-(4-bromophenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one (**3**) (10 mmol) in refluxed methanol (10 ml) or ethanol (10 ml) for 3 h. Compounds **4** or **5** were obtained by cooling the reaction, collecting the precipitate through filtration, washing it with ice water, drying and recrystallizing it from methanol.

Method B In a 250 ml round flask, At room temperature, drops of piperidine were added to a mixture of 10-(4-bromophenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one (**3**) (10 mmol) and malononitrile (10 mmol) in methanol (10 ml) or ethanol (10 ml). After the reaction had advanced for four hours, as verified by TLC, compounds **4** or **5** were obtained by filtering off, drying, and recrystallizing the precipitate from methanol.

6-(4-bromophenyl)-4-(3,4-dimethoxyphenyl)-2-methoxynicotinonitrile (**4**)

Yellow solid, (method A, 265 mg, 62%); (method B, 350 mg, 79%); mp: 190–192 °C. IR (KBr) ν (cm⁻¹) 3447, 3079, 2978, 2943, 2838, 2219, 1581, 1541, 1521, 1449, 1369, 1327, 1253, 1024, 865, 771, 599. ¹H NMR (300 MHz, CDCl₃) δ (ppm) 3.97 (3H, s, OCH₃), 3.99 (3H, s, OCH₃), 4.21 (3H, s, OCH₃), 6.98 (1H, d, $J = 9$ Hz, Ar), 7.27–7.37 (3H, m, Ar), 7.52 (1H, d, $J = 8.7$ Hz, Ar), 7.67–7.69 (3H, m, Ar + CH-pyridine). MS (EI, 70 eV) m/z (%): 425 [M⁺] (25), 410 [M⁺-CH₃] (25), 394 [M⁺-OCH₃] (10), 257 [M⁺-C₉H₁₂O₃] (14). Anal. Calcd. for C₂₁H₁₇BrN₂O₃ (425.28) (%): C, 59.31; H, 4.03; N, 6.59. Found (%): C, 59.37; H, 4.09; N, 6.56.

6-(4-bromophenyl)-4-(3,4-dimethoxyphenyl)-2-ethoxynicotinonitrile (**5**)

Yellow solid; (method A, 279 mg, 65%), (method B, 350 mg, 75%); mp: 190–192 °C. IR (KBr) ν (cm⁻¹) 3447, 2956, 2833, 2221, 1593, 1578, 1540, 1519, 1421, 1343, 1251, 1142, 1039, 1021, 1009, 822, 804, 615. ¹H NMR (300 MHz, DMSO-*d*₆): δ (ppm) 1.45 (3H, t, $J = 7.2$ Hz, CH₃), 3.84 (3H, s, OCH₃), 3.86 (3H, s, OCH₃), 4.63 (2H, q, $J = 7.2$ Hz, CH₂), 7.10 (1H, d, $J = 8.7$ Hz, Ar), 7.66–7.89 (7H, m, Ar + CH-pyridine). ¹³C NMR (DMSO-*d*₆, 75.5 MHz): δ (ppm) 165.1, 157.8, 155.1, 151.3, 149.2, 136.1, 132.0, 129.8, 128.5, 124.4, 120.5, 112.1, 111.1, 110.5, 106.1, 92.1, 63.26, 61.63, 56.0, 55.9, 14.6, 14.3. MS (EI, 70 eV) m/z (%): 438 [M⁺] (100), 423 [M⁺-CH₃]

(27.65), 410 [M⁺-CH₂CH₃] (13.2), 394 [M⁺-OCH₂CH₃] (10), 257 [M⁺-C₉H₁₂O₃] (9). Anal. calcd. for C₂₂H₁₉BrN₂O₃ (439.31) (%): C, 60.15; H, 4.36; N, 6.38. Found (%): C, 60.21; H, 4.40; N, 6.43.

Synthesis of 6-(4-bromophenyl)-4-(3,4-dimethoxyphenyl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile (**7**)

Drops of trimethylamine were added under refluxing temperature to a solution of 3-(4-bromophenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one (**3**) (10 mmol) and cyanothioacetamide (10 mmol) in absolute ethanol (15 mL). The reaction was given time to cool and after a one-hour observation, the orange precipitate was collected by filtration, and the resulting precipitate was dried, and recrystallized from methanol to yield the cyanothiopyridine derivative **7**.

Orange solid; (4 g, 94%); mp: 240–245 °C. IR (KBr) ν (cm⁻¹) 3447, 2050, 2933, 2226 (CN), 1597, 1549, 1518 (C = N), 1489, 1350, 1261, 1144, 1068, 1019, 795. ¹H NMR (300 MHz, CDCl₃) δ (ppm): 3.82–3.98 (6H, m, OCH₃), 6.88–7.16 (2H, m, Ar), 7.26–7.73 (6H, m, Ar + CH-pyridine). MS (EI, 70 eV) m/z (%): 426 [M +] (96), 411[M⁺-NH] (11), 368[M⁺-CSNH] (5). Anal. Calcd. for C₂₀H₁₅BrN₂O₂S (427.31) (%): C, 56.22; H, 3.54; N, 6.56. Found (%): C, 56.30; H, 3.62; N, 6.61.

Synthesis of compounds 9a–c

Compound **7** (10 mmol) was added to the derivatives of 2-chloro-N-phenylacetamide (10 mmol) in 15 ml of refluxing ethanol. Drops of triethylamine were then added, and the reflux was left for two hours. The corresponding S-alkyl pyridine derivatives (**9a–c**) were obtained by filtering off, drying, and recrystallizing the precipitate that was formed after the reaction was allowed to cool.

2-((6-(4-bromophenyl)-3-cyano-4-(3,4-dimethoxyphenyl)pyridin-2-yl)thio)-N-phenylacetamide (**9a**)

Yellow solid; (55 mg, 91%); mp: 230–235 °C. IR (KBr) ν (cm⁻¹) 3313 (NH), 3060, 2967, 2927, 2830, 2213 (CN), 1658 (C = O), 1598 (C = N), 1519, 1375, 1257, 1170, 1133, 821, 757, 964, 495. ¹H NMR (300 MHz, DMSO-*d*₆): δ (ppm) 3.76 (3H, s, OCH₃), 3.79 (3H, s, OCH₃), 4.33 (2H, s, CH₂), 6.84 (1H, d, $J = 8.4$ Hz, Ar), 7.05 (1H, t, $J = 8.0$ Hz, Ar), 7.30 (2H, t, $J = 7.5$ Hz, Ar), 7.57–7.87 (9H, m, Ar + CH-pyridine), 10.30 (1H, s, NH). MS (EI, 70 eV) m/z (%): 559 [M⁺] (40), 469 [M⁺-C₆H₅NH] (46), 439 [M⁺-C₆H₅NHCO] (95), 425 [M⁺-C₆H₅NHCOC] (18). Anal. Calcd. for C₂₈H₂₂BrN₃O₃S (560.46) (%): C, 60.01; H, 3.96; N, 7.50. Found (%): C, 60.11; H, 4.03; N, 7.56.

2-((4-bromophenyl)-3-cyano-4-(3,4-dimethoxyphenyl)pyridin-2-yl)thio-N-(p-tolyl)acetamide (9b)

Yellow solid; (550 mg, 92%); mp: 250–252 °C. IR (KBr) ν (cm^{-1}) 3241 (NH), 3048, 2931, 2834, 2215 (CN), 1652 (C=O), 1598 (C=N), 1517, 1376, 1259, 1137, 1020, 819, 503. ^1H NMR (300 MHz, DMSO- d_6): δ (ppm) 2.25 (3H, s, CH₃), 3.77 (3H, s, OCH₃), 3.79 (3H, s, OCH₃), 4.31 (2H, s, CH₂), 6.85 (1H, d, $J = 8.7$ Hz, Ar), 7.10 (2H, d, $J = 8.1$ Hz, Ar), 7.46 (2H, d, $J = 8.4$ Hz, Ar), 7.69 (2H, d, $J = 8.4$ Hz, Ar), 7.67–7.87 (5H, m, Ar + CH-pyridine), 10.19 (1H, s, NH). MS (EI, 70 eV) m/z (%): 573 [M^+] (30), 467 [M^+ -CH₃C₆H₅NH] (38), 438 [M^+ -CH₃C₆H₅NHCO] (86), 425 [M^+ -CH₃C₆H₅NHCOC] (16), 393 [M^+ -FC₆H₅NHCOCS] (2.5). Anal. Calcd. for C₂₉H₂₄BrN₃O₃S (574.49) (%): C, 60.63; H, 4.21; N, 7.31. Found (%): C, 60.68; H, 4.25; N, 7.36.

2-((4-bromophenyl)-3-cyano-4-(3,4-dimethoxyphenyl)pyridin-2-yl)thio-N-(4-fluorophenyl)acetamide (9c)

Yellow solid; (510 mg, 87%); mp: 259–262 °C. IR (KBr) ν (cm^{-1}) 3434, 3257 (NH), 3056, 2935, 2836, 2215 (CN), 1654 (C=O), 1598 (C=N), 1513, 1376, 1259, 1218, 1133, 1074, 821, 511. ^1H NMR (300 MHz, DMSO- d_6) δ (ppm) 3.77 (3H, s, OCH₃), 3.80 (3H, s, OCH₃), 4.32 (2H, s, CH₂), 6.88 (1H, d, $J = 8.4$ Hz, Ar), 7.14 (2H, t, $J = 8.7$ Hz, Ar), 7.56–7.61 (2H, m, Ar), 7.65–7.71 (2H, m, Ar), 7.77–7.88 (5H, m, Ar + CH-pyridine), 10.35 (1H, s, NH). ^{13}C NMR (100 MHz, DMSO- d_6) δ (ppm) 166.2, 164.6, 162.4, 158.7, 157.6, 155.0, 153.6, 153.4, 151.9, 149.5, 147.3, 135.6, 132.4, 131.3, 129.6, 124.3, 121.9, 121.5, 121.4, 121.3, 116.0, 115.9, 115.7, 112.0, 111.4, 56.3, 56.1, 35.4. MS (EI, 70 eV) m/z (%): 579 [M^+] (50), 467 [M^+ -FC₆H₅NH] (50.8), 439 [M^+ -FC₆H₅NHCO] (91), 425 [M^+ -FC₆H₅NHCOC] (16), 393 [M^+ -FC₆H₅NHCOCS] (16). Anal. Calcd. for C₂₈H₂₁BrFN₃O₃S (578.46) (%): C, 58.14; H, 3.66; N, 7.26. Found (%): C, 58.14; H, 3.66; N, 7.32.

Synthesis of the thienopyridine derivatives (10a–c)

Method A To a round flask containing sodium ethoxide solution, for three hours, compounds **9a–c** (10 mmol) were refluxed. In all cases, a yellow precipitate was seen. After allowing the reactions to cool, the precipitates were removed by filtering, drying, and recrystallizing in the appropriate solvent to yield the corresponding thieno[2,3-*b*]pyridine-2-carboxamide (**10a–c**).

Method B Compound **7** (10 mmol) was refluxed in sodium ethoxide solution for three hours along with the appropriate derivative of 2-chloro-*N*-phenylacetamide (10 mmol). A yellow precipitate was observed and allowed to cool. The

precipitate was then filtered off, dried, and crystallized from the appropriate solvent to yield the corresponding thieno[2,3-*b*]pyridine-2-carboxamide derivatives (**10a–c**).

3-amino-6-(4-bromophenyl)-4-(3,4-dimethoxyphenyl)-*N*-phenylthieno[2,3-*b*]pyridine-2-carboxamide (10a)

Yellow solid; (method A, 45 mg, 83%), (method B, 40 mg, 71%); mp: 230–232 °C. IR (KBr) ν (cm^{-1}) 3477–3397 (NH₂), 3318 (NH), 3081, 3000, 2927, 2829, 1631 (C=O), 1594 (C=N), 1517, 1482, 1432, 1317, 1238, 1027, 802, 752, 690, 499, 422. ^1H NMR (300 MHz, DMSO- d_6) δ (ppm) 3.82 (3H, s, OCH₃), 3.87 (3H, s, OCH₃), 6.01 (2H, br, NH₂), 7.02–7.10 (2H, m, Ar), 7.31 (2H, t, $J = 7.8$ Hz, Ar), 7.53 (2H, d, $J = 8.1$ Hz, Ar), 7.68–7.78 (7H, m, Ar + CH-pyridine), 9.41 (1H, br, NH). ^{13}C NMR (DMSO- d_6 , 75.5 MHz) δ (ppm) 164.1, 160.1, 155.9, 150.8, 149.2, 146.8, 146.2, 138.9, 135.9, 131.7, 131.0, 130.2, 128.5, 123.7, 123.5, 122.9, 121.6, 120.5, 120.3, 120.2, 117.9, 111.9, 110.6, 98.4, 55.9, 55.7. MS (EI, 70 eV) m/z (%): 561 [M^+] (81), 467 [M^+ -C₆H₅NH] (100), 439 [M^+ -C₆H₅NHCO] (3.2). Anal. Calcd. for C₂₈H₂₂BrN₃O₃S (560.46) (%): C, 60.01; H, 3.96; N, 7.50. Found (%): C, 60.08; H, 4.02; N, 7.55.

3-Amino-6-(4-bromophenyl)-4-(3,4-dimethoxyphenyl)-*N*-(p-tolyl)thieno[2,3-*b*]pyridine-2-carboxamide (10b)

Yellow solid; (method A, 540 mg, 94%), (method B, 45 mg, 76%); mp: 140–142 °C. IR (KBr) ν (cm^{-1}) 3473 (NH₂), 3316 (NH), 3079, 2996, 2829, 1633 (C=O), 1592 (C=N), 1513, 1398, 1315, 1238, 1027, 806, 505. ^1H NMR (300 MHz, CDCl₃) δ (ppm): 2.32 (3H, s, CH₃), 3.93 (3H, s, OCH₃), 3.99 (3H, s, OCH₃), 5.82 (2H, s, NH₂), 6.92 (1H, d, $J = 8.4$ Hz, Ar), 7.12 (2H, d, $J = 8.1$ Hz, Ar), 7.37–7.43 (6H, m, Ar), 7.57–7.75 (4H, m, Ar + CH-pyridine + NH). ^{13}C NMR (DMSO- d_6 , 75.5 MHz) δ (ppm) 163.7, 159.8, 155.5, 150.5, 148.9, 146.1, 145.8, 136.5, 135.8, 132.2, 131.4, 130.8, 130.0, 128.6, 122.6, 121.5, 120.4, 120.1, 117.6, 111.7, 110.4, 98.8, 55.6, 55.4, 20.3. MS (EI, 70 eV) m/z (%): 573 [M^+] (98), 467 [M^+ -C₇H₁₀N] (100), 439 [M^+ -C₈H₈NO] (8), 367 [M^+ -C₁₀H₁₀N₂OS] (3). Anal. Calcd. for C₂₉H₂₄BrN₃O₃S (574.49) (%): C, 60.63; H, 4.21; N, 7.31. Found (%): C, 60.65; H, 4.27; N, 7.38.

3-amino-6-(4-bromophenyl)-4-(3,4-dimethoxyphenyl)-*N*-(4-fluorophenyl)thieno[2,3-*b*]pyridine-2-carboxamide (10c)

Yellow solid; [A] (530 mg, 90%). [B] (50 mg, 75%); mp: 205–207 °C. IR (KBr) ν (cm^{-1}) 3531–3484 (NH₂), 3324 (NH), 3077, 2965, 2836, 1635 (C=O), 1596 (C=N), 1509, 1400, 1315, 1240, 1012, 829, 809, 636, 514, 493. ^1H NMR (300 MHz, DMSO- d_6) δ (ppm) 3.95 (3H, s, OCH₃),

4.01 (3H, s, OCH₃), 5.90 (2H, s, NH₂), 6.94–7.07 (3H, m, Ar), 7.21–7.27 (1H, m, Ar), 7.39–7.52 (5H, m, Ar), 7.60–7.76 (4H, m, Ar + CH-pyridine + NH). MS (EI, 70 eV) *m/z* (%): 579 [M⁺] (76), 467 [M⁺-FC₆H₅NH] (100), 439 [M⁺-FC₆H₅NHCO] (3), 367 [M-C₉H₇FN₂OS] (3). Anal. Calcd. for C₂₈H₂₁BrFN₃O₃S (578.45) (%): C, 58.14; H, 3.66; N, 7.26. Found (%): C, 58.1; H, 3.61; N, 7.55.

Synthesis of compounds 11a–c

Method A A solution of **7** (10 mmol) and the suitable α -chloroketones ethyl chloroacetate, phenacyl chloride, or chloroacetone (10 mmol) in absolute ethanol (15 mL) was prepared by adding drops of triethylamine. The mixture was refluxed for 4 h and then allowed to cool. The precipitate that was formed was filtered off, dried, and crystallized from the appropriate solvent to yield the corresponding derivatives of compounds **11a–c**, respectively.

Method B After 10 h of vigorous stirring in DMF (15 mL) containing **7** (10 mmol), the appropriate α -chloroketones (10 mmol), and potassium hydroxide (10 mmol), the pale yellow product in each case was filtered off, dried, and crystallized from methanol to yield the S-alkylated pyridine derivatives (**11a–c**).

Ethyl 2-((6-(4-bromophenyl)-3-cyano-4-(3,4-dimethoxyphenyl)pyridin-2-yl)thio)acetate (11a)

Yellow solid; (method A, 45 mg, 90%), (method B, 38 mg, 67%); mp: 165–167 °C. IR (KBr) ν (cm⁻¹) 3447, 3081, 2970, 2935, 2677, 2207 (CN), 1735 (C = O), 1569 (C = N), 1569, 1517, 1413, 1267, 1148, 1023, 815. ¹H NMR (300 MHz, DMSO-*d*₆) δ (ppm) 1.11 (3H, t, *J* = 6.9 Hz, CH₃), 3.85 (3H, s, OCH₃), 3.90 (3H, s, OCH₃), 4.08 (2H, q, *J* = 6.9 Hz, CH₂), 4.28 (2H, s, CH₂), 7.09 (1H, d, *J* = 8.4 Hz, Ar), 7.68–7.91 (6H, m, Ar), 8.06 (1H, s, CH-pyridine). MS (EI, 70 eV) *m/z* (%): 514.22 [M⁺] (81), 484[M⁺-CH₂CH₃] (7), 467 [M⁺-CH₂CH₃O] (100), 439 [M⁺-C₃H₅O₂] (3), 394 [M⁺-C₄H₇O₂S] (4). Anal. Calcd. for C₂₄H₂₁BrN₂O₄S (513.41) (%): C, 56.15; H, 4.12; N, 5.46. Found (%): C, 56.21; H, 4.11; N, 5.55.

6-(4-bromophenyl)-4-(3,4-dimethoxyphenyl)-2-((2-oxo-2-phenylethyl)thio)-nicotinonitrile (11b)

Yellow solid; (method A, 55 mg, 94%), (method B, 50 mg, 89%); mp: 201–202 °C. IR (KBr) ν (cm⁻¹) 3064, 2998, 2937, 2908, 2838, 2211 (CN), 1675 (C = O), 1596 (C = N), 1565, 1517, 1411, 1265, 1143, 1016, 829, 811, 757, 692, 644, 570. ¹H NMR (500 MHz, DMSO-*d*₆) δ (ppm) 3.57 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 4.86 (2H, s, CH₂), 6.80 (1H, d, *J* = 8.4 Hz, Ar), 7.41–7.54 (7H, m, Ar + CH-

pyridine), 7.62–7.70 (3H, m, Ar + CH-pyridine), 8.05 (2H, d, *J* = 8.4 Hz, Ar). ¹³C NMR (100 MHz, DMSO-*d*₆) δ (ppm) 193.1, 162.1, 158.4, 153.6, 151.8, 149.4, 136.3, 135.5, 134.2, 132.4, 131.4, 129.4, 128.9, 127.0, 124.3, 121.5, 116.3, 115.9, 114.2, 111.95, 110.9, 110.8, 56.1, 55.6, 38.7. MS (EI, 70 eV) *m/z* (%): 544 [M⁺] (25), 439 [M⁺-C₇H₅O] (55), 393 [M⁺-C₈H₇OS] (2), 105 [M⁺-C₂₁H₁₆BrN₂O₂S] (100). Anal. Calcd. for C₂₈H₂₁BrN₂O₃S (545.45) (%): C, 61.66; H, 3.88; N, 5.14. Found (%): C, 61.71; H, 3.95; N, 5.16.

6-(4-bromophenyl)-4-(3,4-dimethoxyphenyl)-2-((2-oxopropyl)thio)nicotinonitrile (11c)

Yellow solid; (method A, 45 mg, 91%), (method B, 39 mg, 80%); mp: 185–187 °C. IR (KBr) ν (cm⁻¹) 3430, 3085, 3002, 2938, 2210 (CN), 1718 (C = O), 1596 (C = N), 1569, 1521, 1375, 1359, 1257, 1137, 1018, 819, 578, 505. ¹H NMR (300 MHz, CDCl₃) δ (ppm) 2.36 (3H, s, CH₃), 3.97 (3H, s, OCH₃), 4.05 (2H, s, CH₃), 4.06 (3H, s, OCH₃), 6.97 (1H, d, *J* = 8.4 Hz, Ar), 7.47–7.71 (7H, m, Ar + CH-pyridine). MS (EI, 70 eV) *m/z* (%): 484 [M⁺] (45), 467 [M⁺-CH₃] (7), 439 [M⁺-C₂H₅O] (100), 425 [M⁺-C₃H₅O] (13), 393 [M⁺-C₃H₅OS] (2). Anal. Calcd. for C₂₃H₁₉BrN₂O₃S (483.38) (%): C, 57.15; H, 3.96; N, 5.80. Found (%): C, 57.22; H, 4.01; N, 5.87.

Synthesis of compounds 12a–c

Method A For three to six hours, compounds **11a–c** (10 mmol) were refluxed in a sodium ethoxide solution. In each case, the mixtures were cooled to precipitate a yellow solid, which was subsequently filtered off, dried, and crystallized from the appropriate solvent to yield the corresponding thieno[2,3-*b*]pyridine derivatives (**12a–c**).

Method B Compound **7** (10 mmol) was refluxed in sodium ethoxide solution for three hours along with the appropriate α -haloketone derivatives, including ethyl chloroacetate, phenacyl chloride, or chloroacetone (10 mmol). The corresponding thieno[2,3-*b*]pyridine derivatives (**12a–c**) were then obtained by filtering off, drying, and recrystallizing the precipitate that had formed after the reaction was allowed to cool (**12a–c**).

Ethyl 3-amino-6-(4-bromophenyl)-4-(3,4-dimethoxyphenyl)thieno[2,3-*b*]pyridine-2-carboxylate (12a)

Yellow solid; (method A, 45 mg, 90%), (method B, 40 mg, 76%); mp: 218–222 °C. IR (KBr) ν (cm⁻¹) 3487–3354 (NH₂), 3078, 2994, 2829, 1660 (C = O), 1599 (C = N), 1516, 1484, 1402, 1351, 1248, 1141, 1011, 800, 767, 638. ¹H NMR (300 MHz, DMSO-*d*₆) δ (ppm) 1.29 (3H, t,

$J = 6.9$ Hz, CH₃), 3.83 (3H, s, OCH₃), 3.88 (3H, s, OCH₃), 4.27 (2H, q, $J = 6.9$ Hz, CH₂), 5.48 (2H, s, NH₂), 7.04 (1H, d, $J = 9.3$ Hz, Ar), 7.49–7.81 (7H, m, Ar + CH-pyridine). MS (EI, 70 eV) m/z (%): 514 [M⁺] (91), 484[M⁺-CH₂CH₃] (7), 467 [M⁺-CH₂CH₃O] (41), 439 [M⁺-C₃H₅O₂] (3), 397 [M⁺-C₅H₇NO₂] (4). Anal. Calcd. for C₂₄H₂₁BrN₂O₄S (513.40) (%): C, 56.15; H, 4.12; N, 5.46. Found (%): C, 56.21; H, 4.15; N, 5.52.

(3-amino-6-(4-bromophenyl)-4-(3,4-dimethoxyphenyl)thieno[2,3-b]pyridin-2-yl)(phenyl)methanone (12b)

Yellow solid; (method A, 55 mg, 95%), (method B, 35 mg, 65%); mp: 275–278 °C. IR (KBr) ν (cm⁻¹) 3473–3286 (NH₂), 3083, 2948, 2927, 2825, 1596 (C = N), 1535, 1513, 1457, 1428, 1324, 1255, 1022, 732, 701, 653, 593, 404. ¹H NMR (300 MHz, DMSO-*d*₆): δ (ppm) 3.81 (3H, s, OCH₃), 3.84 (3H, s, OCH₃), 6.93–7.00 (3H, m, Ar), 7.53 (4H, d, $J = 7.8$ Hz, Ar), 7.72–7.78 (8H, m, Ar + CH-pyridine + NH₂). ¹³C NMR (DMSO-*d*₆, 75.5 MHz): δ (ppm) 189.0, 162.4, 157.2, 151.1, 150.4, 149.2, 147.4, 140.9, 135.6, 132.0, 131.2, 130.9, 129.8, 128.5, 127.5, 123.2, 121.4, 120.8, 120.7, 119.1, 117.9, 111.9, 110.6, 103.7, 55.8. MS (EI, 70 eV) m/z (%): 546 [M⁺] (97), 529 [M⁺-NH₂] (10), 105 [M⁺-C₂₁H₁₆BrN₂O₂S] (12). Anal. Calcd. for C₂₈H₂₁BrN₂O₃S (545.45) (%): C, 61.66; H, 3.88; N, 5.14. Found (%): C, 61.65; H, 3.98; N, 5.16.

1-(3-amino-6-(4-bromophenyl)-4-(3,4-dimethoxyphenyl)thieno[2,3-b]pyridin-2-yl)ethan-1-one (12c)

Yellow solid; (method A, 43 mg, 90%), (method B, 35 mg, 73%); mp: 255–257 °C. IR (KBr) ν (cm⁻¹) 3480–3311 (NH₂), 3085, 2996, 2931, 2830, 1614 (C = O), 1531 (C = N), 1463, 1247, 1139, 1024, 673, 572, 501, 414. ¹H NMR (300 MHz, DMSO-*d*₆): δ (ppm) 2.37 (3H, s, CH₃), 3.83 (3H, s, OCH₃), 3.87 (3H, s, OCH₃), 6.56 (2H, br, NH₂), 7.08 (1H, d, $J = 8.4$ Hz, Ar), 7.53–7.60 (2H, m, Ar), 7.78–7.85 (5H, m, Ar + CH-pyridine). MS (EI, 70 eV) m/z (%): 484 [M⁺] (92), 467 [M⁺-CH₃] (7), 439 [M⁺-CH₃CO] (3). Anal. Calcd. for C₂₃H₁₉BrN₂O₃S (483.38) (%): C, 57.15; H, 3.96; N, 5.80. Found (%): C, 57.22; H, 4.04; N, 5.85.

Synthesis of compounds 13 and 14

To a round flask, Triethylamine drops were added to a mixture of 10 mmol of compound 7, 10 mmol of methyl iodide or 10 mmol of chloro acetonitrile in 15 mL of absolute ethanol. After three hours of reflux, the mixture was allowed to cool. Compounds **13** and **14** were obtained by filtering off, drying, and crystallizing the precipitate that had formed in each case using the appropriate solvent.

6-(4-bromophenyl)-4-(3,4-dimethoxyphenyl)-2-(methylthio)nicotinonitrile (13)

Yellow solid; (40 mg, 91%); mp: 198–201 °C. IR (KBr) ν (cm⁻¹) 3068, 2999, 2969, 2918, 2819, 2208 (CN), 1590 (C = N), 1568, 1519, 1414, 1358, 1272, 1172, 1150, 1023, 808, 584, 572, 474, 458. ¹H NMR (300 MHz, CDCl₃): δ (ppm) 2.72 (3H, s, CH₃), 3.81 (3H, s, OCH₃), 3.83 (3H, s, OCH₃), 6.98 (1H, d, $J = 9.5$ Hz, Ar), 7.42 (1H, s, Ar), 7.51 (2H, d, $J = 9.1$ Hz, Ar), 7.66–7.75 (4H, m, Ar + CH-pyridine). ¹³C NMR (100 MHz, DMSO-*d*₆): δ (ppm) 181.9, 163.5, 153.3, 152.7, 151.9, 149.6, 149.6, 135.6, 132.4, 131.9, 131.4, 130.8, 129.7, 124.2, 121.6, 115.4, 112.4, 111.2, 102.3, 56.2, 13.6. MS (EI, 70 eV) m/z (%): 442 [M⁺] (56), 425 [M⁺-CH₃] (16), 393 [M⁺-CH₃S] (3). Anal. Calcd. for C₂₁H₁₇BrN₂O₂S (441.34) (%): C, 57.15; H, 3.88; N, 6.35. Found (%): C, 57.22; H, 3.92; N, 6.43.

6-(4-bromophenyl)-2-((cyanomethyl)thio)-4-(3,4-dimethoxyphenyl)nicotinonitrile (14)

Yellow solid; (40 mg, 88%); mp: 165–167 °C. IR (KBr) ν (cm⁻¹) 3432, 3089, 2991, 2931, 2211 (CN), 1592 (C = N), 1571, 1517, 1415, 1270, 1145, 1020, 817, 572, 470. ¹H NMR (300 MHz, CDCl₃): δ (ppm) 3.98 (3H, s, OCH₃), 4.02 (3H, s, 2OCH₃), 4.07 (2H, s, CH₂), 7.00 (1H, d, $J = 8.4$ Hz, Ar), 7.50–7.55 (3H, m, Ar), 7.62–7.73 (3H, m, Ar+ CH-pyridine), 7.86 (1H, s, Ar). MS (EI, 70 eV) m/z (%): 467 [M⁺] (52), 450 [M⁺-CH₃] (23), 386 [M⁺-Br] (15). Anal. Calcd. for C₂₂H₁₆BrN₃O₂S (466.35) (%): C, 56.66; H, 3.46; N, 9.01. Found (%): C, 56.74; H, 3.51; N, 9.09.

Preparation of 3-amino-6-(4-bromophenyl)-4-(3,4-dimethoxyphenyl)thieno[2,3-b]pyridine-2-carboxamide (15)

To obtain compound **15**, compound **14** (10 mmol) was refluxed in sodium ethoxide solution for three hours. The reaction was then allowed to cool, and the precipitate that was formed was filtered off, dried, and crystallized from methanol.

Yellow solid; (35 mg, 80%); mp: 235–238 °C. IR (KBr) ν (cm⁻¹) 3482, 3380, 3087 (2NH₂), 2829, 1641 (C = O), 1585 (C = N), 1403, 1353, 1251, 1025, 580, 759, 647, 559, 449, 416. ¹H NMR (300 MHz, DMSO-*d*₆): δ (ppm) 3.82 (3H, s, OCH₃), 3.87 (3H, s, OCH₃), 5.90 (2H, s, NH₂), 7.03 (1H, d, $J = 8.4$ Hz, Ar), 7.18 (2H, s, NH₂), 7.51 (2H, d, $J = 7.5$ Hz, Ar), 7.72–7.76 (5H, m, Ar + CH-pyridine). ¹³C NMR (DMSO-*d*₆, 75.5 MHz): δ (ppm) 166.9, 159.5, 155.3, 150.5, 148.9, 145.9, 145.5, 135.8, 131.4, 130.8, 130.0, 122.5, 120.3, 120.1, 117.5, 111.7, 110.3, 98.4, 55.6, 55.5. MS (EI, 70 eV) m/z (%): 485 [M⁺] (95), 467 [M⁺-NH₂] (78), 439 [M⁺-CONH₂] (14). Anal. Calcd. for

C₂₂H₁₈BrN₃O₃S (484.37) (%): C, 54.55; H, 3.75; N, 8.68. Found (%): C, 54.58; H, 3.80; N, 8.71.

Antimicrobial activity

Media and microorganisms

Modified nutrient agar medium slants (g/L) at pH 7.0 were used to sustain *Escherichia coli*, *Bacillus mycoides*, and *Candida albicans*: Peptone: 3, Yeast extract: 1.5, Meat extract: 1.5, Glucose: 0.5, NaCl: 0.25, and Agar: 20.0. The microbial strains were seeded and grown at 37 °C on nutrient agar medium (70148 Nutrient agar, Fluka, Spain) with the following components (g/L): peptone, 5.0; yeast extract, 2.0; meat extract, 1.0; sodium chloride, 5.0; and agar, 15.0. This was done to conduct the agar diffusion method for antimicrobial assessment, where 28 g of the ready medium was dissolved in 1.0 L of distilled water to create the nutrient agar medium at pH 7.0. Autoclaving was used to sterilize the aforementioned media for 20 min at 121 degrees Celsius while using 1.5 atmospheres.

Assessment of antimicrobial activity

Utilizing the agar diffusion technique, the effectiveness of the produced compounds as antibacterial agents was evaluated against three representative microorganisms [34]. Using 100 µl (1.5 × 10⁵ CFU/100 µl) of re-suspended overnight culture at 37 °C, the microbial cultures were seeded in the nutrient agar medium (70148 Nutrient agar, Fluka) as follows: The specified microbial inoculums were added to the melting medium at around 45 °C before the nutrient agar media was poured onto a series of Petri dishes. At room temperature, the plates were allowed to harden before 200 µl of dissolved substances (10 mg/mL in DMSO) were administered to 15 mm holes made in the infected agar plates. Standard antibiotic disks (7 mm) (Ampicillin 10 µg, Gentamicin 10 µg, and Tobramycin 10 µg) from Bioanalyse[®] were also used for comparison with the tested compounds. The test samples were serially diluted in DMSO to achieve concentrations ranging from 5 to 0.0048 mg/mL in order to establish the minimal inhibitory concentration (MIC). The MIC was established as the lowest concentration that could prevent observable microbial growth [27, 38, 39]. Following a 24-h incubation period at 37 °C, the established inhibitory zones on culture plates were examined [40].

Data availability

Data supporting the productivity of this investigation are available from the corresponding author upon request.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1007/s00044-024-03188-1>.

Author contributions FMS and MAE Supervision, Project administration, Conceptualization, Chemical Reactions, Formal analysis, Investigation of the chemical compounds. FMS Writing-original draft, Writing-review & editing. AME Investigation, Data curation, antimicrobial activity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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