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ORIGINAL PAPER

Synthetic utility of bifunctional thiophene derivatives and antimicrobial evaluation of the newly synthesized agents

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Abstract The key intermediate ethyl 2-amino-5-benzoyl-4-methylthiophene-3-carboxylate was prepared by a Gewald reaction starting from benzoylacetone, sulfur, and ethyl cyanoacetate in the presence of diethylamine. This intermediate reacted with various reagents to afford different fused and polyfunctional substituted thiophenes. Antimicrobial screening of the synthesized compounds exhibited promising antimicrobial activities.

Keywords Ethyl thiophene-3-carboxylate · Thienopyrimidines · Thienopyridines · Tetrazole derivatives · Pyrazole derivatives · Antimicrobial activity

Introduction

Ethyl 2-aminothiophene-3-carboxylate represents as a versatile synthetic building block for the preparation of many condensed heterocyclic systems, viz. tetrazole, azapyrazole, pyridine, and pyrimidine ring systems [1], especially when linked to a pyrimidine ring as thienopyrimidines show potent analgesic [2], anti-inflammatory [3–5], antipyretic [6], antimicrobial [7, 8], anticonvulsant [9], and antiplatelet activities [10, 11], and other central nervous system (CNS) activities [12].

In addition, the pyrimidine nucleus can be found in a broad variety of antibacterial, anticancer, and antitumor agents as well as in agrochemicals and veterinary products [13]. In continuation of our interest in the development of new and simple methods for the synthesis of polyfunctional substituted heterocycles and thienopyrimidines with anticipated biological activity [14–17], the present work describes the syntheses and antimicrobial activities of some condensed heterocyclic thiophenes and thienopyrimidines.

Results and discussion

Ethyl 2-amino-5-benzoyl-4-methylthiophene-3-carboxylate (**1**) was prepared by the Gewald reaction [18] of benzoylacetone, sulfur, and ethyl cyanoacetate in the presence of diethylamine and used as a starting material. Its IR spectrum displayed absorption bands at 3,652 (NH_2), 1,690, and 1,620 cm^{-1} (the last two signals attributed to two CO groups), and absence of the CN group. The ^1H NMR spectrum showed two signals at $\delta = 1.32$ and 4.30 ppm for the ester protons and an exchangeable broad signal at $\delta = 6.55$ ppm for the two protons of the amino group. The ^{13}C NMR spectrum showed two CO signals at $\delta = 161.2$ and 167.9 ppm for the ester and benzoyl carbonyl groups.

Treatment of **1** with triethyl orthoformate and sodium azide afforded ethyl 5-benzoyl-4-methyl-2-(1*H*-tetrazol-1-yl)thiophene-3-carboxylate (**2**) in good yield [19]. The ^1H NMR spectrum of **2** showed a singlet signal at $\delta = 8.85$ ppm characteristic of a tetrazole proton, and a lack of the amino proton signal detected for the parent

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1; moreover the mass spectrum of **2** showed the ion peak at $m/z = 342$ (M^+) in accordance with the molecular weight ($C_{16}H_{14}N_4O_3S$).

2,3-Diamino-6-benzoyl-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (**3**) was obtained by refluxing **2** with hydrazine hydrate. The reaction sequence and mechanism are outlined in Scheme 1 [19–21]. The ^{1}H NMR spectrum of **3** revealed two exchangeable broad singlet signals at $\delta = 6.41$ and 11.49 ppm for two amino groups without the ester proton signals detected for the parent **2**. Moreover, the mass spectrum of **3** showed the ion peak at $m/z = 300$ (M^+) in accordance with the molecular weight ($C_{14}H_{12}N_4O_2S$). It is noteworthy that when compound **1** was hydrolyzed in an aqueous ethanolic solution of sodium hydroxide, the corresponding acid **4** [22] was obtained in fairly good yield, which on treatment with triethyl orthoformate and sodium azide yielded 5-benzoyl-4-methyl-2-(1*H*-tetrazol-1-yl)thiophene-3-carboxylic acid (**5**). The ^{1}H NMR spectrum of **5** showed a signal at $\delta = 8.89$ ppm characteristic of a tetrazole proton and at $\delta = 12.1$ ppm for the carboxylic proton (Scheme 1).

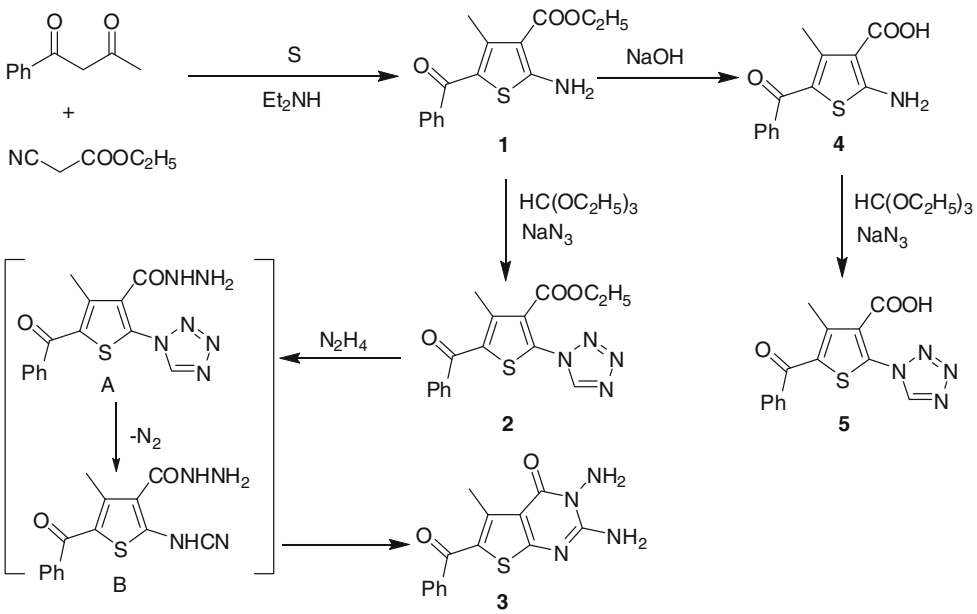
Compound **1** reacted with benzoyl isothiocyanate in ethanolic sodium hydroxide to afford a cyclized product, 6-benzoyl-2,3-dihydro-5-methyl-2-thioxothieno[2,3-*d*]pyrimidin-4(1*H*)-one (**6**) [23, 24]. Its ^{1}H NMR spectrum showed signals at $\delta = 9.52$ and 11.21 ppm (exchangeable NH protons), with the other protons in their expected location and a lack of the signals attributed to the NH_2 protons and ester protons detected in the parent **1**. The ^{13}C NMR spectrum showed the CS signal at $\delta = 168.7$ ppm beside the two CO signals at $\delta = 162.1$ and 168.1 ppm. The mass spectrum had an ion peak at $m/z = 302$ (M^+) in accordance with the molecular weight ($C_{14}H_{10}N_2O_2S_2$).

Also, when compound **1** reacted with phenyl isothiocyanate or phenyl isocyanate in the presence of a catalytic amount of triethylamine, the corresponding thioureidothiophene and ureidothiophene derivatives **7a** and **7b** were afforded, respectively, which underwent cyclization in ethanolic sodium ethoxide to yield the thieno[2,3-*d*]-pyrimidine derivatives **8a** and **8b**, respectively. The ^{1}H NMR spectrum of **8b** showed an exchangeable signal at $\delta = 9.32$ ppm corresponding to the NH group (Scheme 2).

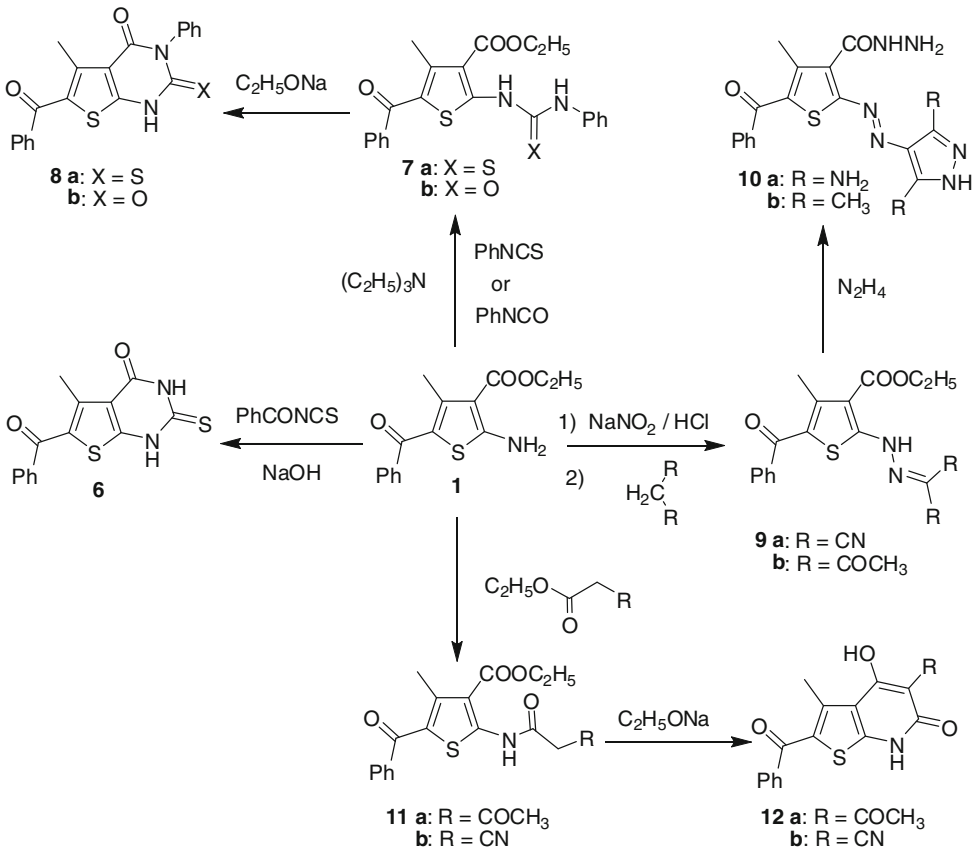
Compound **1** was diazotized with hydrochloric acid and sodium nitrite. The desired diazonium chloride was then coupled with some active methylene compounds, namely malononitrile and acetylacetone to yield the corresponding azo derivatives **9a** and **9b**, respectively [25, 26]. The ^{1}H NMR spectrum of **9b** showed the absence of an amino signal and the presence of signals at $\delta = 2.34$ and 2.35 ppm for two acetyl groups and $\delta = 13.0$ ppm for an exchangeable NH proton. Hydrazones **9a** and **9b** reacted with hydrazine hydrate in ethanol under reflux to afford the hydrazides **10a** and **10b**. The ^{1}H NMR spectrum of **10b** exhibited signals at $\delta = 6.90$ (exchangeable NH_2 protons) and 9.10 and 11.50 ppm (exchangeable NH protons). Moreover, its mass spectrum showed the ion peak at $m/z = 382$ (M^+) in accordance with the molecular weight ($C_{18}H_{18}N_6O_2S$).

When **1** was thermally fused with ethyl cyanoacetate or ethyl acetoacetate in the presence of a catalytic amount of piperidine **11a** and **11b**, respectively, were formed. Furthermore, compounds **11a** and **11b** underwent cyclization in refluxing ethanolic sodium ethoxide to afford the corresponding thieno[3,2-*b*]pyridine-2-one derivatives **12a** and **12b**, respectively. The NMR data supported the assignment of structures of **11a**, **11b**, **12a**, and **12b**.

Scheme 1



Scheme 2



Antimicrobial screening

Antibacterial activity

All the synthesized compounds were evaluated for their in vitro antibacterial activity against *Escherichia coli*, a gram-negative bacteria, and *Staphylococcus aureus* and *Bacillus cereus*, which are gram-positive bacteria, using the cup plate method [27, 28]. The activities were compared with those of the known standard drug tavanic (used at 0.001 mol/cm^3). The zone of inhibition (mm) of each compound against the microorganisms is listed in Table 1. The inhibition exhibited by compounds **2**, **3**, **6**, **8a**, **8b**, **10a**, and **10b** was more effective than that of the other novel compounds for all bacterial strains. Moreover, these compounds exhibited fairly good activity, comparable to that of the standard tavanic. Other compounds were either fairly or moderately active against the tested bacterial strains. The enhanced antibacterial activity of the compounds is attributed to the presence of tetrazole, pyrimidine, and pyrazole moieties of the thiophene derivatives.

Antifungal activity

Antifungal activity of the compounds was determined against *Aspergillus niger* and *Candida albicans* using the cup plate method, and the activities were compared with those of the known standard drug nystatin (used at 0.001 mol/cm^3). All compounds showed good to excellent activity against the tested fungi. In particular, compounds **2**, **3**, **6**, **8a**, **8b**, **10a**, and **10b** exhibited even stronger activity than nystatin against the tested fungi. The antimicrobial test results imply that in general thienopyrimidine derivatives exhibit higher activities than the corresponding thiophenes against human pathogenic bacteria and phytopathogenic fungi.

Conclusion

We have developed rapid and effective procedures for the synthesis of the polycondensed heterocyclic ring systems. The compounds **2**, **3**, **6**, **8a**, **8b**, **10a**, and **10b** exhibited high antimicrobial activity. The structure–activity relationship suggested that heterocyclization reactions of bifunctional thiophenes to tetrazole, pyrazole, and pyrimidine derivatives can result in products with high antibacterial and antifungal activity. Our prediction is that these compounds may show even better antimicrobial activity.

Experimental

All melting points were measured on an Electrothermal IA 9100 series digital melting point apparatus. Microanalytical

Table 1 Antimicrobial activity of the new compounds

Sample	Inhibition of zone diameter (mm)				
	Antibacterial activity			Antifungal activity	
	E. coli	*S. aureus*	*B. cereus*	*Candida albicans*	*Aspergillus niger*
1	6	5	9	8	12
2	22	23	24	23	27
3	24	26	27	26	30
4	9	8	11	10	14
5	18	20	17	19	23
6	23	24	26	24	28
7a	17	17	19	18	22
7b	15	14	17	16	20
8a	21	22	23	22	26
8b	20	21	22	23	25
9a	16	15	18	17	21
9b	13	12	15	14	18
10a	18	18	20	19	23
10b	19	20	21	20	24
11a	11	10	13	12	16
11b	14	13	16	15	19
12a	10	9	12	11	15
12b	12	11	14	13	17
Tavanic	30	26	28	–	–
Nystatin	–	–	–	27	26
DMF	+ve	+ve	+ve	+ve	+ve

Less active (2–5 mm); moderately active (6–14 mm); highly active (15–30 mm); very highly active (>30 mm). +ve indicates growth of microbes. Control *N*,*N*-dimethylformamide (DMF) (0.01% solution in distilled water). Standard for antibacterial: tavanic (0.001 mol/cm^3). Standard for antifungal: nystatin (0.001 mol/cm^3). Inhibition was recorded by measuring the diameter of the inhibition zone at the end of 24 h for bacteria and for 48 h for fungi

data were carried out in the microanalytical center, Faculty of Science, Cairo University. The IR spectra (KBr disk) were recorded using a Perkin-Elmer 1650 spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Jeol JMS-AX 500 (^{1}H, 500 MHz; ^{13}C, 125.76 MHz) with Me_4Si as internal standard. Mass spectra were recorded on an EI MS-QP 1000 EX (Shimadzu, Japan) at 70 eV. The pharmacological evaluations were carried out in the pharmacological unit, Department of Chemistry of Natural and Microbial Products, National Research Center, Egypt.

Ethyl 2-amino-5-benzoyl-4-methylthiophene-3-carboxylate (**1**, $C_{15}H_{15}NO_3S$)

A mixture of benzoylacetone (10 mmol), ethyl cyanoacetate (10 mmol), sulfur (10 mmol), and diethylamine (10 mmol) was heated at 70 °C with stirring in 20 cm^3 absolute ethanol for 4 h. The reaction mixture was left overnight in a refrigerator at 0 °C. The solid product was collected by filtration, washed with ethanol, dried, and recrystallized from ethanol to afford **1** (85%). M.p.: 215–217 °C; IR (KBr): $\bar{\nu}$ = 3,652 (NH_2), 1,690, 1,620 (2 CO) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.32 (t, J = 6.9 Hz, 3H, CH_3), 2.22 (s, 3H, CH_3), 4.30 (q, 2H, J = 7.2 Hz, CH_2), 6.55 (br.s, 2H, NH_2), 7.44–7.94 (m, 5H, Ar–H) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 23.1, 24.3, 39.8, 40.1, 40.2, 115.3, 116.8, 119.5, 125.6, 129.1, 143.7, 161.2, 167.9 ppm; MS: m/z (%) = 289 (33) [M$^+$].

Ethyl 5-benzoyl-4-methyl-2-(1H-tetrazol-1-yl)thiophene-3-carboxylate (**2**, $C_{16}H_{14}N_4O_3S$)

A mixture of **1** (10 mmol), triethyl orthoformate (10 mmol), and sodium azide (10 mmol) in 40 cm^3 glacial acetic acid was stirred under reflux for 2 h. The reaction mixture was cooled and suspended in 7 cm^3 conc. HCl. The solid was collected by suction filtration and washed with water. The crude product was recrystallized from ethanol to afford **2** (70%). M.p.: 243–245 °C; IR (KBr): $\bar{\nu}$ = 1,695, 1,618 (2 CO) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.31 (t, 3H, J = 6.9 Hz, CH_3), 2.23 (s, 3H, CH_3), 4.29 (q, 2H, J = 7.3 Hz, CH_2), 7.45–7.95 (m, 5H, Ar–H), 8.85 (s, 1H, tetrazole H-5) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 20.2, 23.5, 60.2, 110.2, 128.7, 129.6, 132.5, 133.3, 138.3, 143.2, 144.4, 149.5, 162.1, 166.7 ppm; MS: m/z (%) = 342 (27) [M$^+$].

2,3-Diamino-6-benzoyl-5-methylthieno[2,3-d]pyrimidin-4(3H)-one (**3**, $C_{14}H_{12}N_4O_2S$)

Compound **2** (10 mmol) in 15 cm^3 hydrazine hydrate was heated under reflux for 7 h. The reaction mixture was cooled and suspended in 50 cm^3 water. The solid was collected by suction filtration, washed with water, and recrystallized from ethanol to afford **3** (75%). M.p.: 270–272 °C; IR (KBr): $\bar{\nu}$ = 3,400, 3,385 (2 NH_2), 1,650, 1,622 (2 CO), 1,585 (C=N) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.20 (s, 3H, CH_3), 6.41 (br.s, 2H, CNH_2), 7.43–7.94 (m, 5H, Ar–H), 11.49 (br.s, 2H, NNH_2) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 19.2, 121.3, 128.6, 129.3, 131.5, 132.3, 135.3, 142.2, 146.4, 149.5, 158.1, 168.9 ppm; MS: m/z (%) = 300 (60) [M$^+$].

5-Benzoyl-4-methyl-2-(1H-tetrazol-1-yl)thiophene-3-carboxylic acid (**5**, $C_{14}H_{10}N_4O_3S$)

Compound **5** was obtained from **4** in a manner similar to that described for **2** (yield 75%). M.p.: 292–294 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.24 (s, 3H, CH_3), 7.45–7.93 (m, 5H, Ar–H), 8.89 (s, 1H, tetrazole H-5), 12.10 (br.s, 1H, COOH) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 23.2, 108.2, 124.2, 126.7, 130.5, 131.3, 138.3,

134.6, 138.5, 144.4, 151.3, 164.6, 172.5 ppm; MS: *m/z* (%) = 314 (31) [M^+].

6-Benzoyl-2,3-dihydro-5-methyl-2-thioxothieno-[2,3-d]pyrimidin-4(1H)-one (**6**, $C_{14}H_{10}N_2O_2S_2$)
A mixture of **1** (10 mmol) and benzoyl isothiocyanate (10 mmol) in ethanolic NaOH (0.4 g in 40 cm^3) was refluxed for 6 h. The reaction mixture was cooled and suspended in 6 cm^3 conc. HCl. The solid was filtered, washed with water, and recrystallized from ethanol to afford **6** (70%). M.p.: 180–182 °C; IR (KBr): $\bar{\nu}$ = 3,360, 3,310 (2 NH), 1,665, 1,630 (2 CO), 1,575 (C=N) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.23 (s, 3H, CH_3), 7.44–7.95 (m, 5H, Ar–H), 9.55 (br.s, 2H, NH), 11.21 (s, 1H, NH) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 22.9, 25.7, 39.7, 40.2, 115.9, 118.1, 124.9, 126.8, 127.1, 129.4, 129.8, 138.6, 143.3, 162.1, 168.1, 168.7 ppm; MS: *m/z* (%) = 302 (15) [M^+].

*General procedure for preparation of compounds **7a**, **7b***

A mixture of compound **1** (10 mmol), phenyl isothiocyanate or phenyl isocyanate (10 mmol), and 1 cm^3 triethylamine in 40 cm^3 dioxane was reflux for 8 h. The solution was evaporated to $\approx 1/3$ of its volume; the separated precipitate was filtered, washed with ethanol, dried, and recrystallized from dioxane to afford **7a** and **7b**, respectively.

Ethyl 5-benzoyl-4-methyl-2-(3-phenylthioureido)thiophene-3-carboxylate (**7a**, $C_{22}H_{20}N_2O_3S_2$)
Yield 60%; m.p.: 236–238 °C; IR (KBr): $\bar{\nu}$ = 3,370, 3,360 (2 NH), 1,710, 1,670 (2 CO) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.32 (t, 3H, J = 6.8 Hz, CH_3), 2.25 (s, 3H, CH_3), 4.28 (q, 2H, J = 7.2 Hz, CH_2), 6.73–7.94 (m, 10H, Ar–H), 8.64 (s, 1H, NH), 9.10 (s, 1H, NH) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 19.4, 23.5, 59.6, 104.9, 125.1, 126.8, 127.8, 128.4, 129.6, 130.8, 132.9, 137.7, 140.1, 157.1, 161.9, 174.3, 184.6 ppm; MS: *m/z* (%) = 424 (65) [M^+].

Ethyl 5-benzoyl-4-methyl-2-(3-phenylureido)-thiophene-3-carboxylate (**7b**, $C_{22}H_{20}N_2O_4S$)
Yield 70%; m.p.: 250–252 °C; IR (KBr): $\bar{\nu}$ = 3,370, 3,365 (2 NH), 1,712, 1,685, 1,673 (3 CO) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.31 (t, 3H, J = 6.8 Hz, CH_3), 2.23 (s, 3H, CH_3), 4.29 (q, 2H, J = 7.3 Hz, CH_2), 7.01–7.95 (m, 10H, Ar–H), 8.59 (s, 1H, NH), 9.45 (s, 1H, NH) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 19.5, 22.1, 58.3, 110.3, 119.4, 124.2, 126.2, 127.5, 128.7, 129.1, 131.6, 132.2, 133.0, 143.3, 150.5, 154.2, 163.6, 168.6 ppm; MS: *m/z* (%) = 408 (23) [M^+].

*General procedure for preparation of compounds **8a**, **8b***

Compound **7a** or **7b** (10 mmol) in sodium ethoxide (0.23 g of sodium metal in 40 cm^3 ethanol) was stirred under reflux for 8 h. After cooling, the mixture was neutralized with 10 cm^3 10% HCl and the solid formed was filtered, washed with water, dried, and recrystallized from ethanol to afford **8a**, **8b**.

6-Benzoyl-2,3-dihydro-5-methyl-3-phenyl-2-thioxothieno[2,3-d]pyrimidin-4(1H)-one
(**8a**, $C_{20}H_{14}N_2O_2S_2$)
Yield 70%; m.p.: 277–279 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.25 (s, 3H, CH_3), 7.00–7.96 (m, 10H, Ar–H), 9.20 (s, 1H, NH) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 19.2, 109.4, 119.4, 125.9, 126.7, 127.4, 128.9, 130.5, 131.8, 132.5, 133.4, 135.6, 140.3, 155.7, 170.4, 182.3 ppm; MS: *m/z* (%) = 378 (71) [M^+].

6-Benzoyl-5-methyl-3-phenylthieno[2,3-d]pyrimidine-2,4(1H,3H)-dione (**8b**, $C_{20}H_{14}N_2O_3S$)
Yield 75%; m.p.: 285–287 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.26 (s, 3H, CH_3), 7.05–7.97 (m, 10H, Ar–H), 9.32 (s, 1H, NH) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 18.9, 109.8, 119.8, 124.8, 126.4, 128.2, 128.8, 130.7, 131.6, 132.3, 134.8, 135.2, 148.1, 152.0, 154.4, 166.9 ppm; MS: *m/z* (%) = 362 (41) [M^+].

*General procedure for preparation of compounds **9a**, **9b***

A solution of compound **1** in conc. HCl (2 mmol in 5 cm^3) was kept in an ice bath at 0–5 °C for 10 min. An aqueous solution of sodium nitrite (2.1 mmol in 5 cm^3) was added dropwise with stirring to the amine hydrochloride salt solution over a period of 20–25 min at 0 °C. A yellow precipitate of diazonium hydrochloride salt was formed. The reaction mixture was stirred for additional 15 min while maintaining the temperature at 0 °C. Malononitrile (2 mmol) or acetylacetone (2.2 mmol) was added to a solution of the amine hydrochloride salt and 5 g anhydrous sodium acetate in 100 cm^3 ethanol with stirring at 0–5 °C. Stirring was continued for an additional 3 h. The mixture was left overnight in the refrigerator. Water (250 cm^3) was added to the reaction mixture and the solid product was collected by filtration and recrystallized from ethanol to afford **9a**, **9b**.

Ethyl 5-benzoyl-2-[2-(dicyanomethylene)-hydrazinyl]-4-methylthiophene-3-carboxylate
(**9a**, $C_{18}H_{14}N_4O_3S$)
Yield 60%; m.p.: 190–192 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.35 (t, 3H, J = 6.9 Hz, CH_3), 2.22 (s, 3H, CH_3), 4.30 (q, 2H, J = 7.4 Hz, CH_2), 7.44–7.94 (m,

5H, Ar–H), 12.60 (s, 1H, NH) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 17.2, 20.3, 54.3, 94.9, 103.4, 111.7, 119.3, 128.7, 130.3, 131.5, 133.1, 137.2, 144.3, 162.4, 171.5 ppm; MS: *m/z* (%) = 366 (28) [M^+].

Ethyl 2-[2-(1-acetyl-2-oxopropylidene)hydrazinyl]-5-benzoyl-4-methylthiophene-3-carboxylate (**9b**, $C_{20}H_{20}N_2O_5S$)
Yield 70%; m.p.: 175–177 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.30 (t, 3H, J = 6.8 Hz, CH_3), 2.29 (s, 3H, CH_3), 2.34 (s, 3H, $COCH_3$), 2.35 (s, 3H, $COCH_3$), 4.31 (q, 2H, J = 7.3 Hz, CH_2), 7.45–7.95 (m, 5H, Ar–H), 13.00 (s, 1H, NH) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 22.2, 23.7, 25.1, 31.9, 39.2, 77.2, 112.4, 115.4, 126.3, 128.8, 129.2, 129.3, 130.5, 134.8, 142.6, 160.9, 167.3, 171.1, 173.2 ppm; MS: *m/z* (%) = 400 (20) [M^+].

*General procedure for preparation of compounds **10a, 10b***

A mixture of **9a** or **9b** (10 mmol) and hydrazine hydrate (15 mmol) in 30 cm^3 ethanol was heated under reflux for 6 h. The solid precipitated after concentration was filtered, dried, and recrystallized from ethanol to afford **10a**, **10b**.

2-(3,5-Diamino-1H-pyrazol-4-ylazo)-5-benzoyl-4-methylthiophene-3-carboxylic acid hydrazide (**10a**, $C_{16}H_{16}N_8O_2S$)
Yield 55%; m.p.: 257–259 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.22 (s, 3H, CH_3), 2.65, 2.80, 4.43 (3br.s, 6H, $3NH_2$), 7.43–7.93 (m, 5H, Ar–H), 9.15 (br.s, 1H, NH), 11.55 (br.s, 1H, NH) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 19.7, 120.6, 125.4, 128.7, 129.4, 130.6, 131.4, 133.7, 134.9, 139.1, 140.2, 146.0, 163.3, 168.7 ppm; MS: *m/z* (%) = 384 (30) [M^+].

5-Benzoyl-2-(3,5-dimethyl-1H-pyrazol-4-ylazo)-4-methylthiophene-3-carboxylic acid hydrazide (**10b**, $C_{18}H_{18}N_6O_2S$)
Yield 70%; m.p.: 238–240 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.24 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.82 (s, 3H, CH_3), 6.90 (br.s, 2H, NH_2), 7.44–7.94 (m, 5H, Ar–H), 9.10 (br.s, 1H, NH), 11.50 (br.s, 1H, NH) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 12.3, 119.1, 124.2, 126.5, 128.4, 130.1, 131.5, 133.3, 135.2, 138.9, 141.7, 144.5, 148.3, 162.8, 166.8 ppm; MS: *m/z* (%) = 382 (70) [M^+].

*General procedure for preparation of compounds **11a, 11b***

A mixture of **1** (10 mmol), ethyl acetoacetate or ethyl cyanoacetate (10 mmol), and few drops of piperidine were thermally fused for 5 h. The reaction mixture was cooled to room temperature and the solid formed was collected by filtration, washed with ether, and recrystallized from ethanol to afford **11a** and **11b**, respectively.

Ethyl 5-benzoyl-4-methyl-2-(3-oxobutyrylamino)thiophene-3-carboxylate (**11a**, $C_{19}H_{19}NO_5S$)
Yield 65%; m.p.: >300 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.30 (t, 3H, J = 6.9 Hz, CH_3), 2.23 (s, 3H, CH_3), 2.30 (s, 3H, $COCH_3$), 3.35 (s, 2H, CH_2) 4.31 (q, 2H, J = 7.2 Hz, CH_2), 7.44–7.94 (m, 5H, Ar–H), 9.75 (br.s, 1H, NH) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 22.2, 38.9, 39.2, 39.4, 39.7, 77.2, 112.4, 115.4, 126.3, 128.8, 129.2, 129.3, 130.5, 142.6, 160.9, 167.3, 171.1, 173.2 ppm; MS: *m/z* (%) = 373 (44) [M^+].

Ethyl 5-benzoyl-2-(2-cyanoacetylamino)-4-methylthiophene-3-carboxylate (**11b**, $C_{18}H_{16}N_2O_4S$)
Yield 55%; m.p.: 295–297 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 1.32 (t, 3H, J = 7.0 Hz, CH_3), 2.24 (s, 3H, CH_3), 3.36 (s, 2H, CH_2), 4.32 (q, 2H, J = 7.4 Hz, CH_2), 7.45–7.95 (m, 5H, Ar–H), 9.80 (br.s, 1H, NH) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 14.6, 18.7, 27.2, 60.1, 110.8, 124.3, 127.4, 129.9, 130.5, 131.5, 135.1, 137.0, 140.6, 152.4, 163.7, 169.6 ppm; MS: *m/z* (%) = 356 (62) [M^+].

*General procedure for preparation of compounds **12a, 12b***

A solution of **11a** or **11b** (10 mmol) in sodium ethoxide (0.23 g of sodium metal in 40 cm^3 ethanol) was stirred under reflux for 9 h. After cooling the reaction mixture was neutralized with cooled 10% HCl and the solid formed was collected by filtration, washed with water, dried, and recrystallized from ethanol to afford **12a** and **12b**, respectively.

5-Acetyl-2-benzoyl-4-hydroxy-3-methylthieno[2,3-b]-pyridin-6(7H)-one (**12a**, $C_{17}H_{13}NO_4S$)
Yield 60%; m.p.: >300 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.23 (s, 3H, CH_3), 2.32 (s, 3H, $COCH_3$), 7.44–7.95 (m, 5H, Ar–H), 10.65 (br.s, 1H, NH), 12.25 (br.s, 1H, OH) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 22.2, 38.9, 39.2, 39.7, 77.28, 112.4, 115.4, 126.3, 128.8, 129.2, 129.3, 130.5, 142.6, 160.9, 167.3, 171.0, 173.1 ppm; MS: *m/z* (%) = 327 (25) [M^+].

2-Benzoyl-6,7-dihydro-4-hydroxy-3-methyl-6-oxothieno[2,3-b]pyridine-5-carbonitrile (**12b**, $C_{16}H_{10}N_2O_3S$)
Yield 70%; m.p.: >300 °C; IR (KBr): $\bar{\nu}$ = 3,522 (OH), 3,377 (NH), 2,225 (CN), 1,680, 1,637 (2 CO) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.22 (s, 3H, CH_3), 7.43–7.96 (m, 5H, Ar–H), 10.60 (br.s, 1H, NH), 12.20 (br.s, 1H, OH) ppm; MS: *m/z* (%) = 310 (54) [M^+].

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