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Synthesis and antimicrobial activity of novel polycyclic thienopyridazine derivatives

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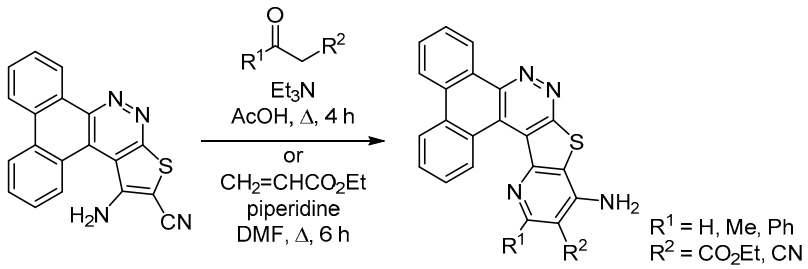


A series of novel thienopyridazine derivatives has been synthesized using 9-aminodibenzo[*f*,*h*]thieno[2,3-*c*]cinnoline-8-carbonitrile as starting material. The target products exhibit promising antibacterial and antifungal properties against bacterial strains *E. coli*, *S. aureus*, *B. subtilis*, and *K. pneumoniae* and fungal strains *A. fumigatus* and *C. albicans*. The highest antimicrobial activity against all tested microorganisms demonstrate pyridothienocinnoline derivatives.

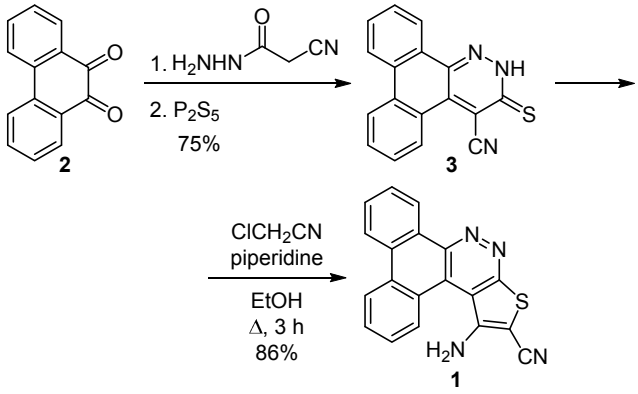
Keywords: pyridothienocinnoline, pyrimidothienocinnoline, thiazinothienocinnoline, thiazolopyrimidothienocinnoline, thienocinnoline, thienopyridazine, triazolopyrimidothienocinnoline, antimicrobial agent.

Pyridazine and pyridazinone derivatives have attracted increased interest as inhibitors of glycogen synthase kinase 3 (GSK-3)[1] and acetylcholinesterase (AChE),[2] antagonists of histamine H3 receptor (H3R), and compounds with antisecretory, gastric antiulcer,[3] antihypertensive,[4] and cardiovascular-related[5] activity. In addition, several thienopyridazines and pyridazinones have been developed as potent anticancer agents,[6] new allosteric modulators of adenosine A_1 receptor (A_1AR),[7] and inhibitors of NAD(P)H oxidase.[8] Based on these reports and our previous research regarding synthesis of pyrazole, pyridine, pyrimidine, and thienopyrimidine derivatives and evaluation of their biological activities,[9] we present herein preparation and assessment of antimicrobial activity of novel thienopyridazines (thienocinnolines).

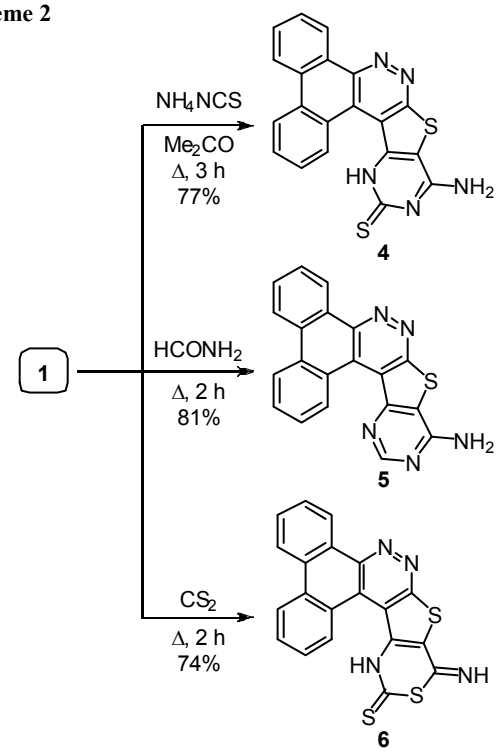
A series of thienopyridazine derivatives were obtained using 9-aminodibenzo[*f*,*h*]thieno[2,3-*c*]cinnoline-8-carbonitrile (**1**). The synthesis commenced with preparation of starting material **1**. Reaction of phenanthrene-9,10-dione (**2**) and 2-cyanoacetohydrazide followed by thionylation with P_2S_5 afforded 3-thioxo-2,3-dihydrodibenzo[*f*,*h*]cinnoline-4-carbonitrile (**3**) in 75% yield.[10] Further cyclization of intermediate **3** with chloroacetonitrile in presence of piperidine in EtOH provided compound **1** in 86% yield (Scheme 1).

Reaction of 9-aminodibenzo[*f*,*h*]thieno[2,3-*c*]cinnoline-8-carbonitrile (**1**) and ammonium isothiocyanate or formamide afforded pyrimidothienocinnoline derivatives **4** and **5**, respectively. Similarly, treatment of compound **1** with carbon disulfide allowed to obtain thiazinothienocinnoline derivative **6** (Scheme 2). Furthermore, reaction of

Scheme 1

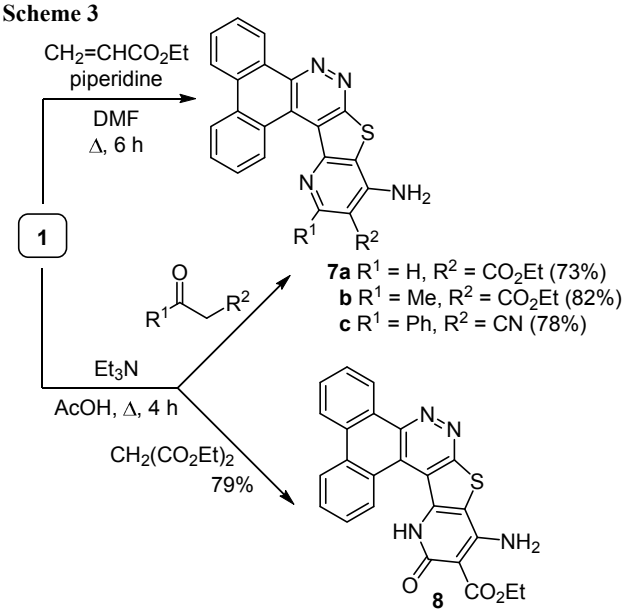


Scheme 2



starting material **1** and active methylene compounds (ethyl acetoacetate, diethyl malonate, or benzoylacetonitrile) or ethyl acrylate furnished pyridothienocinnolines **7a**–**c** and **8** in high yields (Scheme 3).

Scheme 3



Synthesis of pyrimidothienocinnoline derivatives with condensed 1,2,4-triazole and thiazole rings using compound **1** as starting material was also demonstrated. First, reaction of 9-aminodibenzo[*f*,*h*]thieno[2,3-*c*]cinnoline-8-carbonitrile (**1**) and triethyl orthoformate in Ac_2O allowed to obtain ethyl formimidate **9**, which, when treated with hydrazine hydrate, provided the corresponding formimidohydrazide **10**. Further refluxing in Ac_2O–AcOH, 1:3, ensured cyclization of compound **10** and formation of triazolopyrimidothienocinnoline **11** in 75% yield. Ethyl formimidate **9** was also subjected to reaction with mercaptoacetic acid, and thienocinnoline **12** bearing thiazole moiety was obtained. Finally, NaOEt-mediated cyclization of intermediate **12** afforded target product – thiazolopyrimidothienocinnolinone **13** in 72% yield (Scheme 4).

Scheme 4

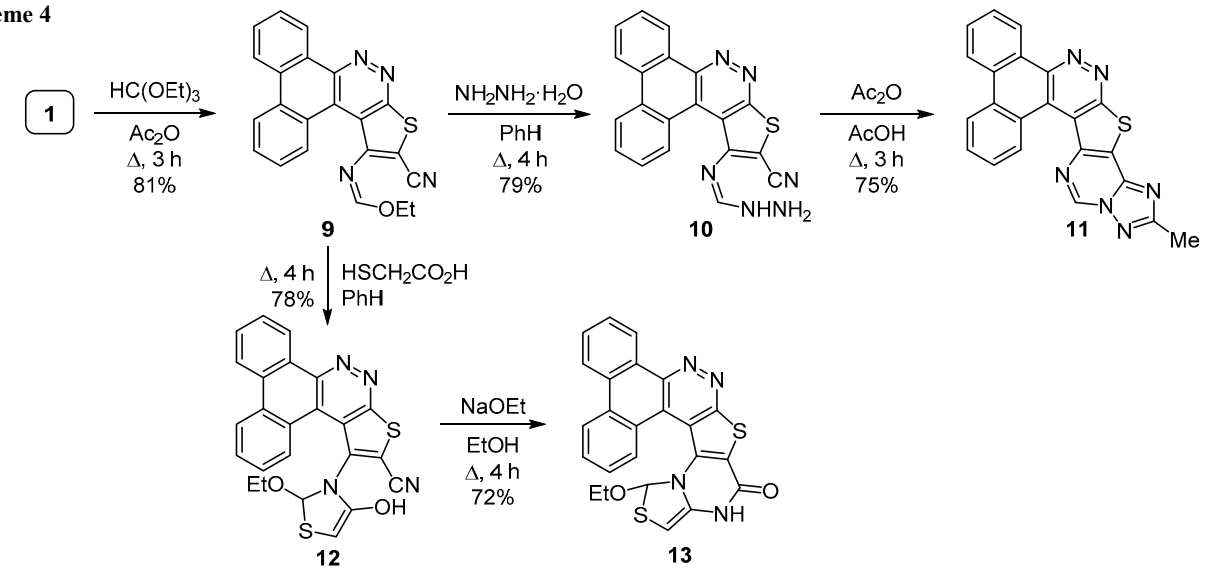


Table 1. Antibacterial and antifungal activity of cinnoline derivatives **4**–**6**, **7a**–**c**, **8**, **11**, **13**, ciprofloxacin, and fusidic acid

Compound**	Diameter of zone of inhibition*, mm					
	Bacterial strain				Fungal strain	
	Escherichia coli	*Staphylococcus aureus*	*Bacillus subtilis*	*Klebsiella pneumoniae*	*Aspergillus fumigatus*	*Candida albicans*
4	15 ± 1	17 ± 2	14 ± 1	19 ± 3	14 ± 1	12 ± 1
5	20 ± 2	19 ± 2	20 ± 1	14 ± 1	15 ± 1	15 ± 2
6	14 ± 2	15 ± 1	13 ± 1	11 ± 2	9 ± 3	21 ± 1
7a	19 ± 1	21 ± 2	20 ± 2	22 ± 3	20 ± 2	19 ± 3
7b	16 ± 2	14 ± 2	17 ± 1	15 ± 1	20 ± 3	13 ± 1
7c	21 ± 1	20 ± 2	21 ± 2	23 ± 2	21 ± 1	22 ± 3
8	17 ± 1	22 ± 1	19 ± 3	17 ± 3	16 ± 3	18 ± 2
11	12 ± 3	14 ± 2	9 ± 1	11 ± 1	6 ± 2	13 ± 1
13	22 ± 1	13 ± 2	7 ± 1	15 ± 3	22 ± 2	9 ± 3
Ciprofloxacin***	23 ± 1	20 ± 1	21 ± 2	20 ± 2	21 ± 1	23 ± 2
Fusidic acid	18 ± 2	14 ± 2	19 ± 3	8 ± 1	11 ± 1	16 ± 3

* ≤15 mm: low inhibition, 16–20 mm: moderate inhibition, ≥20 mm: high inhibition.
** Concentration of tested compounds 10 μg/ml.
*** Concentration of ciprofloxacin 50 μg/ml.

In continuation of this research, *in vitro* antibacterial and antifungal activity of the synthesized novel cinnoline derivatives **4**–**6**, **7a**–**c**, **8**, **11**, and **13** was estimated. The activity assay was carried out using four bacterial strains *(Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Klebsiella pneumoniae)* and two fungal strains (*Aspergillus fumigatus* and *Candida albicans)*. The antibacterial and antifungal activity was evaluated by comparing zone of inhibition for the investigated cinnoline derivatives and two positive control compounds – ciprofloxacin and fusidic acid. The obtained results reveal that the highest antibacterial activity against *E. coli* demonstrates thiazolopyrimidothienocinnolinone **13**, against *S. aureus* – pyridothienocinnoline **8**, and against *B. subtilis* and *K. pneumoniae* – pyridothienocinnoline **7c**. Furthermore, the highest antifungal activity against *A. fumigatus* and *C. albicans* possess compounds **13** and **7c**, respectively. Overall, the highest antibacterial and antifungal activity against all tested microorganisms provide pyridothienocinnolines **7a**,**c**, whereas the lowest – triazolopyrimidothienocinnoline **11** (Table 1).

Minimum inhibitory concentration (MIC) of active cinnoline derivatives **5**, **7c**, and **13** against bacterial strains *E. coli*, *S. aureus*, and *K. pneumoniae* and fungi strain *C. albicans* was also determined. The lowest values of MIC were obtained for compounds **7c** and **13** against *K. pneumoniae* and *S. aureus*, respectively (Table 2).

In conclusion, new thienopyridazine derivatives have been obtained in moderate to high yields using 9-aminodibenzo[*f*,*h*]thieno[2,3-*c*]cinnoline-8-carbonitrile as starting material and applying versatile synthetic methods. The resulting compounds were evaluated as promising antibacterial and antifungal agents against bacterial strains *E. coli*, *S. aureus*, *B. subtilis*, and *K. pneumoniae* and fungal strains *A. fumigatus* and *C. albicans*. Pyridothienocinnoline derivatives provide the highest antibacterial and antifungal activity against all tested microorganisms, whereas compound with triazolopyrimidothienocinnoline core – the lowest.

Table 2. Minimum inhibitory concentration of compounds **5**, **7c**, and **13** against selected bacterial and fungal strains

Microorganism	MIC, μg/ml			
	5	**7c**	**13**	Standard*
Escherichia coli	45	45	45	7.25
Staphylococcus aureus	25	>100	15.5	14.5
Klebsiella pneumoniae	60	12.5	>100	14.5
Candida albicans	>100	>100	30	7.25

* Tetracycline and ketoconazole were used as standard drugs against bacterial and fungal strains, respectively.

Experimental

IR spectra were recorded on a Shimadzu FTIR-8201 spectrometer in KBr pellets. ^{1}H and ^{13}C NMR spectra were acquired on Jeol EX-300 (300 MHz) and Jeol ECA-500 (100 MHz) spectrometers, respectively, in DMSO-d_6 using TMS as internal standard. Mass spectra were recorded on a Thermo Electron Corporation VG 2AM-3F instrument (EI, 70 eV). Elemental analyses were performed on a PerkinElmer 240 elemental analyzer. Melting points were determined on an Electrothermal IA9100 apparatus. Progress of reactions and purity of products were examined by TLC on Merck silica gel 60 F_{254} plates, eluent hexane–CH_2Cl_2, 9:1. Visualization was achieved using UV lamp (254 nm).

Compound **3** was synthesized according to previously reported procedure.[10]

9-Aminodibenzo[*f*,*h*]thieno[2,3-*c*]cinnoline-8-carbonitrile (1). A mixture of 3-thioxo-2,3-dihydrodibenzo[*f*,*h*]-cinnoline-4-carbonitrile (**3**) (287 mg, 1 mmol), chloroacetonitrile (63 μl, 1 mmol), and few drops of piperidine in

EtOH (25 ml) was refluxed for 3 h. After cooling to room temperature, the obtained solid was filtered off and crystallized from MeOH. Yield 280 mg (86%), brown powder, mp 217–219°C. IR spectrum, ν, cm^{-1}: 3295 (NH_2), 2221 (C≡N). ^1H NMR spectrum, δ, ppm: 7.12–7.64 (8H, m, H Ar); 10.41 (2H, s, NH_2). ^{13}C NMR spectrum, δ, ppm: 121.4; 122.3; 124.5; 125.7; 127.1; 129.2; 130.3; 131.5; 132.2; 133.3; 133.4; 136.4; 136.7; 137.5; 138.2; 140.8; 141.2; 142.2; 161.2. Mass spectrum, *m*/*z* (I_{rel}, %): 326 $[M]^+$ (100), 300 $[M–CN]^+$ (45), 284 (17), 228 (32), 204 (12). Found, %: C 69.67; H 2.87; N 17.01. $C_{19}H_{10}N_4S$. Calculated, %: C 69.92; H 3.09; N 17.17.

8-Aminodibenzo[*f*,*h*]pyrimido[4',5':4,5]thieno[2,3-*c*]-cinnoline-10(11*H*)-thione (4). A mixture of compound **1** (326 mg, 1 mmol) and ammonium isothiocyanate (80 mg, 1 mmol) in Me_2CO (20 ml) was refluxed for 3 h. The reaction mixture was then concentrated under reduced pressure. The obtained solid was filtered off and crystallized from EtOH. Yield 297 mg (77%), yellow powder, mp >300°C. IR spectrum, ν, cm^{-1}: 3396–3181 (NH_2, NH), 1158 (C=S). ^1H NMR spectrum, δ, ppm: 6.28 (1H, s, NH); 7.43–7.72 (8H, m, H Ar); 9.58 (2H, s, NH_2). ^{13}C NMR spectrum, δ, ppm: 120.3; 121.8; 123.5; 126.4; 129.4; 130.5; 132.4; 133.2; 133.5; 134.4; 135.2; 137.1; 137.6; 138.7; 140.5; 141.4; 141.7; 144.7; 146.3; 165.3 (C=S). Mass spectrum, *m*/*z* (I_{rel}, %): 385 $[M]^+$ (61), 369 (28), 284 (100), 176 (21). Found, %: C 62.11; H 2.68; N 18.01. $C_{20}H_{11}N_5S_2$. Calculated, %: C 62.32; H 2.88; N 18.17.

Dibenzo[*f*,*h*]pyrimido[4',5':4,5]thieno[2,3-*c*]cinnolin-8-amine (5). A solution of compound **1** (326 mg, 1 mmol) in formamide (20 ml) was refluxed for 2 h. After cooling to room temperature, the obtained solid was filtered off and crystallized from MeOH. Yield 286 mg (81%), brown powder, mp 291–293°C. IR spectrum, ν, cm^{-1}: 3316–3297 (NH_2). ^1H NMR spectrum, δ, ppm: 7.09–7.31 (8H, m, H Ar); 7.42 (1H, s, H pyrimidine); 10.19 (2H, s, NH_2). ^{13}C NMR spectrum, δ, ppm: 121.4; 122.3; 124.5; 125.7; 127.1; 129.2; 130.3; 131.5; 132.2; 133.3; 133.4; 136.4; 136.7; 137.5; 138.2; 140.8; 141.2; 142.2; 143.2; 145.1. Mass spectrum, *m*/*z* (I_{rel}, %): 353 $[M]^+$ (54), 337 (100), 311 (36), 229 (14), 177 (28). Found, %: C 67.75; H 2.95; N 19.61. $C_{20}H_{11}N_5S$. Calculated, %: C 67.97; H 3.14; N 19.82.

8-Imino-8,11-dihydro-10*H*-dibenzo[*f*,*h*][1,3]thiazino-[4',5':4,5]thieno[2,3-*c*]cinnoline-10-thione (6). A solution of compound **1** (326 mg, 1 mmol) in carbon disulfide (15 ml) was refluxed for 2 h. After cooling to room temperature, the reaction mixture was diluted with dry Et_2O (20 ml). The obtained solid was filtered off and crystallized from MeOH. Yield 298 mg (74%), yellow powder, mp >300°C. IR spectrum, ν, cm^{-1}: 3328–3276 (NH), 1163 (C=S). ^1H NMR spectrum, δ, ppm: 6.21 (1H, s, NH thiazine); 7.19–7.72 (8H, m, H Ar); 10.13 (1H, s, NH imine). ^{13}C NMR spectrum, δ, ppm: 121.5; 122.6; 124.4; 125.7; 126.4; 128.8; 130.6; 131.4; 132.6; 134.5; 134.6; 136.6; 136.8; 137.5; 138.6; 140.4; 141.7; 142.5; 145.4; 163.4 (C=S). Mass spectrum, *m*/*z* (I_{rel}, %): 402 $[M]^+$ (23), 326 (100), 284 (46), 200 (39). Found, %: C 59.45; H 2.29; N 13.73. $C_{20}H_{10}N_4S_3$. Calculated, %: C 59.68; H 2.50; N 13.92.

Ethyl 8-aminodibenzo[*f*,*h*]pyrido[2',3':4,5]thieno[2,3-*c*]-cinnoline-9-carboxylate (7a). A mixture of compound **1** (326 mg, 1 mmol), ethyl acrylate (1.0 ml, 10 mmol), and few drops of piperidine in DMF (15 ml) was refluxed for 6 h. After cooling to room temperature, the obtained solid was filtered off and crystallized from AcOH. Yield 310 mg (73%), white powder, mp >300°C. IR spectrum, ν, cm^{-1}: 3314–3191 (NH_2), 1739 (C=O). ^1H NMR spectrum, δ, ppm (*J*, Hz): 1.29 (3H, t, *J* = 7.5, CH_3); 3.16 (2H, q, *J* = 7.5, CH_2); 7.26–7.43 (8H, m, H Ar); 7.51 (1H, s, H pyridine); 10.14 (2H, s, NH_2). ^{13}C NMR spectrum, δ, ppm: 48.3 (CH_3); 51.4 (CH_2); 120.9; 123.5; 125.1; 125.2; 127.5; 129.3; 131.3; 132.4; 133.6; 134.5; 135.1; 138.4; 139.3; 139.4; 140.3; 141.3; 142.3; 143.4; 143.5; 145.3; 166.2 (C=O). Mass spectrum, *m*/*z* (I_{rel}, %): 424 $[M]^+$ (100), 351 $[M–CO_2C_2H_5]^+$ (52), 336 (63), 285 (18), 177 (34). Found, %: C 67.72; H 3.58; N 13.01. $C_{24}H_{16}N_4O_2S$. Calculated, %: C 67.91; H 3.80; N 13.20.

Synthesis of pyridothienocinnolines 7b,c and 8 (General method). A mixture of compound **1** (326 mg, 1 mmol), active methylene compound – ethyl acetoacetate (0.6 ml, 5 mmol), benzoylacetonitrile (726 mg, 5 mmol), or diethyl malonate (0.8 ml, 5 mmol), and few drops of Et_3N in AcOH (20 ml) was refluxed for 4 h. The reaction mixture was then poured into H_2O (20 ml). The obtained solid was filtered off and crystallized from appropriate solvent.

Ethyl 8-amino-10-methyldibenzo[*f*,*h*]pyrido[2',3':4,5]-thieno[2,3-*c*]cinnoline-9-carboxylate (7b). Yield 360 mg (82%), reddish-brown powder, mp >300°C (EtOH). IR spectrum, ν, cm^{-1}: 3100–3017 (NH_2), 1735 (C=O). ^1H NMR spectrum, δ, ppm (*J*, Hz): 1.53 (3H, t, *J* = 7.5, $OCH_2C\underline{H}_3$); 2.26 (3H, s, CH_3); 3.45 (2H, q, *J* = 7.5, $OC\underline{H}_2CH_3$); 7.18–7.54 (8H, m, H Ar); 9.87 (2H, s, NH_2). ^{13}C NMR spectrum, δ, ppm: 26.9 ($OCH_2\underline{C}H_3$); 28.4 (CH_3); 31.2 ($O\underline{C}H_2CH_3$); 120.4; 121.6; 123.9; 124.8; 126.3; 127.7; 130.7; 131.3; 133.5; 134.5; 135.4; 135.6; 136.3; 136.4; 138.4; 140.3; 140.8; 141.3; 142.8; 143.5; 145.4; 165.2 (C=O). Mass spectrum, *m*/*z* (I_{rel}, %): 438 $[M]^+$ (100), 365 $[M–CO_2C_2H_5]^+$ (42), 334 (19), 176 (28). Found, %: C 68.28; H 3.98; N 12.59. $C_{25}H_{18}N_4O_2S$. Calculated, %: C 68.48; H 4.14; N 12.78.

8-Amino-10-phenyldibenzo[*f*,*h*]pyrido[2',3':4,5]thieno-[2,3-*c*]cinnoline-9-carbonitrile (7c). Yield 354 mg (78%), green powder, mp 254–256°C (EtOH). IR spectrum, ν, cm^{-1}: 3264 (NH_2), 2220 (C≡N). ^1H NMR spectrum, δ, ppm: 6.68–7.01 (5H, m, H Ph); 7.12–7.52 (8H, m, H Ar); 9.84 (2H, s, NH_2). ^{13}C NMR spectrum, δ, ppm: 120.3; 121.4; 123.6; 125.3; 125.4; 126.1; 126.3; 128.4; 129.5; 130.1; 130.3; 131.3; 132.1; 133.5; 134.5; 134.6; 137.3; 138.4; 138.6; 140.4; 140.6; 141.4; 142.9; 143.3; 144.4; 162.2. Mass spectrum, *m*/*z* (I_{rel}, %): 453 $[M]^+$ (39), 376 $[M–C_6H_5]^+$ (62), 335 (100), 285 (36), 201 (14). Found, %: C 73.96; H 3.16; N 15.26. $C_{28}H_{15}N_5S$. Calculated, %: C 74.15; H 3.33; N 15.44.

Ethyl 8-amino-10-oxo-10,11-dihydrodibenzo[*f*,*h*]pyrido-[2',3':4,5]thieno[2,3-*c*]cinnoline-9-carboxylate (8). Yield 348 mg (79%), yellow powder, mp 277–279°C (MeOH). IR spectrum, ν, cm^{-1}: 3296–2977 (NH_2, NH), 1739 (C=O

ester), 1667 (C=O amide). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.49 (3H, t, *J* = 7.5, CH_3); 3.28 (2H, q, *J* = 7.5, CH_2); 6.15 (1H, s, NH); 6.92–7.47 (8H, m, H Ar); 10.02 (2H, s, NH_2). ^{13}C NMR spectrum, δ, ppm: 47.4 (CH_3); 52.2 (CH_2); 121.2; 122.6; 124.9; 125.7; 126.4; 129.4; 130.6; 132.1; 133.2; 134.3; 136.3; 136.4; 137.4; 137.5; 139.4; 140.3; 141.2; 142.4; 143.2; 144.6; 164.2 (C=O); 165.4 (C=O). Mass spectrum, *m*/*z* (I_{rel}, %): 440 $[M]^+$ (100), 367 $[M–CO_2C_2H_5]^+$ (62), 284 (12), 200 (18), 176 (32). Found, %: C 65.26; H 3.45; N 12.51. $C_{24}H_{16}N_4O_3S$. Calculated, %: C 65.44; H 3.66; N 12.72.

Ethyl *N*-(8-cyanodibenzo[*f*,*h*]thieno[2,3-*c*]cinnolin-9-yl)formimidate (9). A mixture of compound **1** (326 mg, 1 mmol) and triethyl orthoformate (3.0 ml, 18 mmol) in freshly distilled Ac_2O (20 ml) was refluxed for 3 h. The reaction mixture was then poured into ice water. The obtained solid was filtered off, washed with H_2O (100 ml), air-dried, and crystallized from EtOH. Yield 310 mg (81%), green powder, mp 223–225°C. IR spectrum, ν, cm^{-1}: 2226 (C≡N), 1586 (C=N). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.43 (3H, t, *J* = 7.5, CH_3); 3.21 (2H, q, *J* = 7.5, CH_2); 6.89 (1H, s, CH=N); 7.12–7.47 (8H, m, H Ar). Mass spectrum, *m*/*z* (I_{rel}, %): 382 $[M]^+$ (35), 337 (64), 310 (100), 228 (19). Found, %: C 68.89; H 3.47; N 14.46. $C_{22}H_{14}N_4OS$. Calculated, %: C 69.09; H 3.69; N 14.65.

***N*''-(8-Cyanodibenzo[*f*,*h*]thieno[2,3-*c*]cinnolin-9-yl)-formimidohydrazide (10)**. A suspension of compound **9** (382 mg, 1 mmol) and $NH_2NH_2·H_2O$ (0.5 ml, 1 mmol) in PhH (20 ml) was refluxed for 4 h. After cooling to room temperature, the obtained solid was filtered off, washed with Et_2O (50 ml), air-dried, and crystallized from MeOH. Yield 291 mg (79%), brown powder, mp 246–248°C. IR spectrum, ν, cm^{-1}: 3396–3181 (NH_2, NH), 2221 (C≡N), 1579 (C=N). ^{1}H NMR spectrum, δ, ppm: 6.28 (1H, s, NH); 7.43 (1H, s, CH=N); 7.53–7.72 (8H, m, H Ar); 9.58 (2H, s, NH_2). ^{13}C NMR spectrum, δ, ppm: 120.3; 121.8; 123.5; 124.8; 126.4; 129.4; 130.5; 132.4; 133.2; 133.5; 134.4; 135.2; 137.1; 137.6; 138.7; 140.5; 141.4; 141.7; 144.7; 146.3. Mass spectrum, *m*/*z* (I_{rel}, %): 368 $[M]^+$ (68), 337 (24), 310 (100), 228 (47), 176 (19). Found, %: C 65.02; H 3.07; N 22.59. $C_{20}H_{12}N_6S$. Calculated, %: C 65.20; H 3.28; N 22.81.

9-Methyl-dibenzo[*f*,*h*][1,2,4]triazolo[1'',5'':1',6']pyrimido-[4',5':4,5]thieno[2,3-*c*]cinnoline (11). A suspension of compound **10** (368 mg, 1 mmol) in Ac_2O–AcOH, 1:3 (20 ml) was refluxed for 3 h. After cooling to room temperature, the reaction mixture was diluted with H_2O (20 ml). The obtained solid was filtered off, washed with H_2O (100 ml), air-dried, and crystallized from PhMe. Yield 294 mg (75%), white powder, mp >300°C. IR spectrum, ν, cm^{-1}: 1583 (C=N), 1554 (C=C). ^{1}H NMR spectrum, δ, ppm: 1.84 (3H, s, CH_3); 7.43–7.64 (8H, m, H Ar); 7.72 (1H, s, H pyrimidine). ^{13}C NMR spectrum, δ, ppm: 29.5 (CH_3); 120.3; 121.8; 123.5; 124.8; 126.4; 129.4; 130.5; 132.4; 133.2; 133.5; 134.4; 135.2; 137.1; 137.6; 138.7; 140.5; 141.4; 141.7; 142.3; 144.7; 146.3. Mass spectrum, *m*/*z* (I_{rel}, %): 392 $[M]^+$ (100), 377 $[M–CH_3]^+$ (72), 337 (16), 285 (29), 205 (35). Found, %: C 67.04; H 2.87; N 21.18. $C_{22}H_{12}N_6S$. Calculated, %: C 67.33; H 3.08; N 21.42.

9-(2-Ethoxy-4-hydroxythiazol-3(2*H*)-yl)dibenzo[*f*,*h*]-thieno[2,3-*c*]cinnoline-8-carbonitrile (12). A suspension of compound **9** (382 mg, 1 mmol) and mercaptoacetic acid (70 μl, 1 mmol) in dry PhH (25 ml) was refluxed for 4 h. After cooling to room temperature, the obtained solid was filtered off, washed with hexane (50 ml), air-dried, and crystallized from EtOH. Yield 356 mg (78%), yellow powder, mp 261–263°C. IR spectrum, ν, cm^{-1}: 3335 (OH), 2228 (C≡N). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.38 (3H, t, *J* = 7.5, CH_3); 3.17 (2H, q, *J* = 7.5, CH_2); 5.42 (1H, s, C<u>H</u>OC_2H_5); 7.13 (1H, s, H thiazole); 7.21–7.62 (8H, m, H Ar); 10.24 (1H, s, OH). Mass spectrum, *m*/*z* (I_{rel}, %): 456 $[M]^+$ (47), 310 (100), 284 (21), 228 (18), 200 (38). Found, %: C 63.09; H 3.14; N 12.13. $C_{24}H_{16}N_4O_2S_2$. Calculated, %: C 63.14; H 3.53; N 12.27.

12-Ethoxy-12*H*-dibenzo[*f*,*h*]thiazolo[4'',3'':2',3']-pyrimido[4',5':4,5]thieno[2,3-*c*]cinnolin-8(9*H*)-one (13). A suspension of compound **12** (456 mg, 1 mmol) and NaOEt (1.00 g, 15 mmol) in EtOH (20 ml) was refluxed for 4 h. The reaction mixture was then concentrated under reduced pressure, and the residue was triturated with H_2O (50 ml). The obtained solid was filtered off, washed with H_2O (100 ml), air-dried, and crystallized from MeOH. Yield 329 mg (72%), brown powder, mp >300°C. IR spectrum, ν, cm^{-1}: 3303 (NH), 1660 (C=O). ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.94 (3H, t, *J* = 7.5, CH_3); 3.36 (2H, q, *J* = 7.5, CH_2); 5.18 (1H, s, C<u>H</u>OC_2H_5); 6.61 (1H, s, NH); 7.18 (1H, s, H thiazole); 7.23–7.56 (8H, m, H Ar). ^{13}C NMR spectrum, δ, ppm: 29.3 (CH_3); 31.4 (CH_2); 122.2; 124.2; 125.5; 126.3; 127.6; 129.7; 132.2; 133.3; 135.7; 136.5; 137.1; 137.2; 138.3; 139.3; 140.4; 140.7; 141.3; 141.4; 142.4; 143.4; 144.2; 161.4 (C=O). Mass spectrum, *m*/*z* (I_{rel}, %): 456 $[M]^+$ (100), 353 (39), 283 (14), 227 (32), 175 (43). Found, %: C 63.06; H 3.12; N 12.11. $C_{24}H_{16}N_4O_2S_2$. Calculated, %: C 63.14; H 3.53; N 12.27.

Antimicrobial activity assay. Antimicrobial activity of compounds **4**–**6**, **7a**–**c**, **8**, **11**, and **13** against bacteria *E. coli*, *S. aureus*, *B. subtilis*, and *K. pneumoniae* and fungi *A. fumigatus* and *C. albicans* was determined by agar diffusion method, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS).[11] According to modified Kirby–Bauer disk diffusion method, concentration of the test compounds was 10 μg/ml. Sterile disks containing bacterial strains in Czapek–Dox agar (glucose (10 g), KNO_3 (2 g), K_2HPO_4 (1 g), KCl (0.5 g), $MgSO_4$ (0.5 g), $FeSO_4$ (0.05 g) in distilled H_2O (1 l)) and fungal strains in potato dextrose agar (PDA) (dextrose (4 g) in potatoes extract (1 l)) were impregnated with 10 μg/disk of each test compound. DMSO was used as negative control, and ciprofloxacin and fusidic acid were used as standards. Each tested compound was assayed in triplicate. Calculated average diameters (in mm) of the zone of inhibition for compounds **4**–**6**, **7a**–**c**, **8**, **11**, and **13** were compared with those obtained for standard drugs.

Minimum inhibitory concentration assay. Serial dilution method[12] was applied for determination of MIC of compounds **5**, **7c**, and **13** against bacterial strains *E. coli*, *S. aureus*, and *K. pneumoniae* and fungal strain *C. albicans*. Standard suspension of each microorganism in DMF (10^7 cells/ml, 100 μl) was transferred to plates containing compound **5**, **7c**, or **13** in nutrient broth in a

series of concentrations 6.25, 12.5, 25.0, 50.0, and 100 μg/ml and incubated at 37°C for 24 h. Tetracycline and ketoconazole were used as standard drugs against bacterial and fungal strains, respectively.

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