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Synthesis and antimicrobial activity of 5-hydroxymethyl-8-methyl-2-(*N*-arylimino)-pyrano[2,3-*c*]pyridine-3-(*N*-aryl)-carboxamides

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Abstract—Several novel 2-imino-5-hydroxymethyl-8-methyl-2*H*-pyrano[2,3-*c*]pyridine-3-(*N*-aryl) carboxamides were prepared by reaction of pyridoxal hydrochloride with various *N*-arylcyanoacetamides. Reaction of these compounds with aromatic amines furnished a wide series of 2-(*N*-*R*-phenyl) imino-5-hydroxymethyl-8-methyl-2*H*-pyrano[2,3-*c*]pyridine-3-carboxamides. Antibacterial and antifungal activities of the synthesized compounds were studied. Most of the obtained compounds demonstrated significant activity against bacterial or fungal strains (MIC in the range of 12.5–25 μg/mL), displaying comparable or even better efficacy than the standard drugs.

Variously substituted coumarines (2*H*-2-chromenones), present in the core of many physiologically active agents, display interesting therapeutic properties. In particular, 3-substituted 2-imino derivatives of coumarine have been shown to inhibit several enzymes, as well as to modulate the activity of many receptors. Thus, 2-imino-2*H*-chromene-3-carboxamide **I** was reported as efficient inhibitor of tyrosine kinases, potentially useful as oncolytic agent.[1] Compound **II** was described as promising anti-inflammatory agent.[2] Compound **III** with hidden 2-imino-2*H*-chromene-3-carboxamide fragment possesses potent activity against several tumor cell lines.[3] These examples illustrate the ongoing interest toward new 2-imino-2*H*-chromenes and have prompted us to explore the synthetic route to their heterocycle-modified 7-azaanalogs, which can serve as a promising source of bioactive molecules (see Fig. 1).

In this work, we describe synthesis and biological activity for a series of novel 2-(*N*-aryl) imino-5-hydroxymethyl-8-methyl-pyrano[2,3-*c*]pyridin-3-(*N*-aryl) carboxamides **IV**. These compounds appeared to be potent inhibitors of several pathogenic bacterial and fungal organisms.

There are only a few reported syntheses of 2*H*-pyrano[2,3-*c*]pyridines. Cho et al. described synthesis based on palladium-catalyzed cyclization of iodopyridine allyl ethers.[4] Mekheimer et al.[5] and Stoyanov et al.[6] obtained 2*H*-pyrano[2,3-*c*]pyridines using the reaction of 4-hydroxy-2(1*H*)-pyridone with arylmethylene cyanoacetic esters. Recently, we developed an efficient approach to 2-imino-5-hydroxymethyl-8-methyl-2*H*-pyrano[3,2-*c*]pyridine-3-carboxamides and their 2-imino derivatives.[7] Key reaction, which led to assembly of the target 2*H*-pyrano[2,3-*c*]pyridine heterocyclic system, was interaction of pyridoxal hydrochloride with cyanoacetamide in the presence of piperidine. The procedure is similar to that we recently described for synthesis of substituted 3-carboxamide-2*H*-benzopyran-2-imines,[8–10] and can be recommended as an efficient synthetic approach to their heteroatom-containing analogs, which are useful synthetic intermediates for a variety of further transformations. In this work, using the similar synthetic scheme, we obtained a series of 2*H*-pyrano[3,2-*c*]pyridines **4a**–**j**

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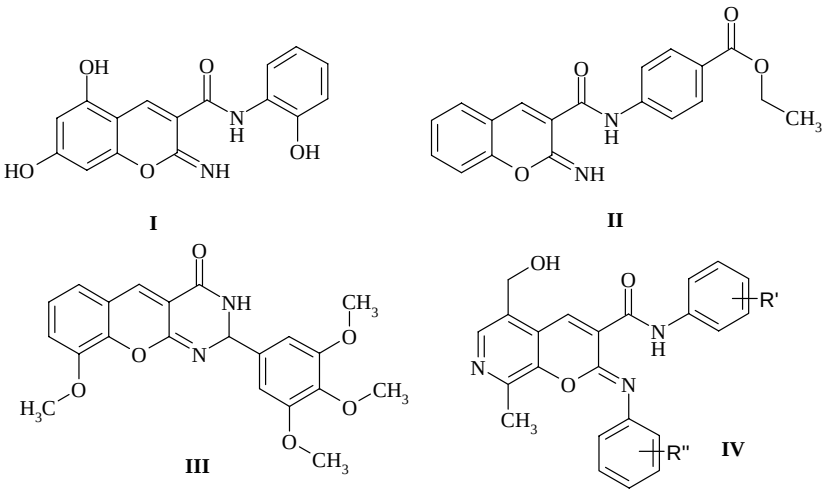
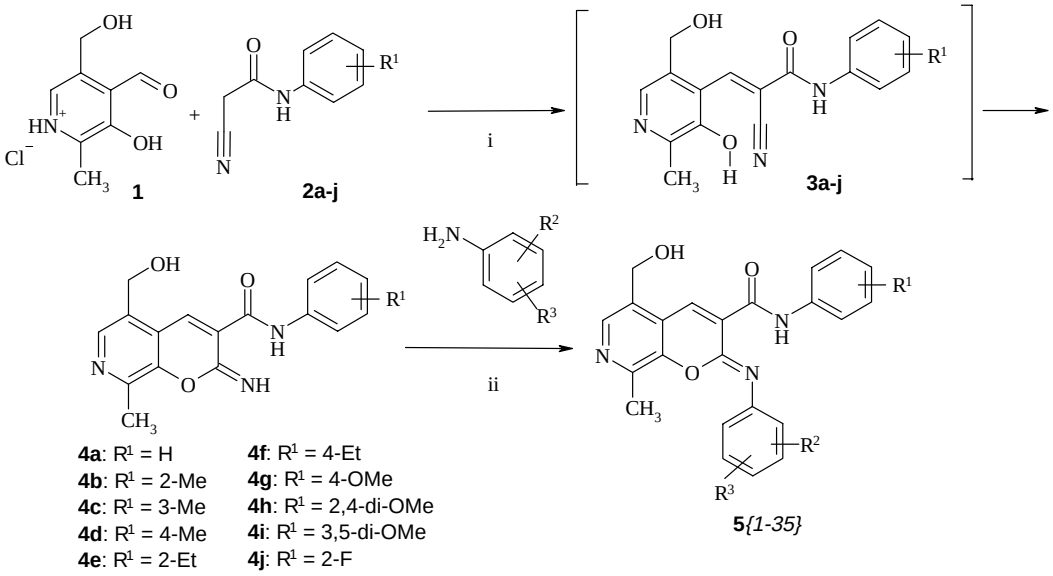


Figure 1. **I–III** Reported biologically active 3-substituted 2-imino-2*H*-chromene derivatives.[1–3] **IV** Structures synthesized in this work.

(Scheme 1, Table 1). Reaction of equimolar amounts of pyridoxal hydrochloride **1** and several substituted 2-cyano-*N*-arylacetamides **2a–j** in the presence of piperidine (2 equiv) afforded arylcarboxamides **4a–j**.[11] The reaction proceeded smoothly in absolute methanol via 2-cyanoacrylamide intermediates **3a–j** and led to assembly of the target 2*H*-pyrano[2,3-*c*]pyridine heterocyclic system. The resulting products precipitated from the reaction mixture. Recrystallization from ethanol gave **4a–j** as white crystals in 56–88% yield.

In the mentioned work,[7] we demonstrated that 5-hydroxymethyl-2-imino-8-methyl-2*H*-pyrano[2,3-*c*]-pyridine-3-carboxamide can readily react with nucleophilic agents, such as $N^{(4)}$-substituted thiosemicarbazides. The reaction proceeded via an interesting intramolecular recyclization mechanism leading to 3-heteroaryl-substituted pyrano[2,3-*c*]pyridin-2-ones. However, in our recent studies we showed that the products of substitution at 2-imino nitrogen can also be formed upon the reaction of 2-iminocoumarin-3-carboxamides with nucleophiles, such as aromatic amines.[8,12] In this work, we observed that their 7-aza analogs can react with aromatic amines in a similar manner. Reaction of **4a–j** with several substituted anilines in boiling acetic acid led to formation of *N*-arylimino derivatives **5**{*1–35*}.[13] The target compounds usually crystallized on cooling to room temperature and could be isolated from the reaction mixture by filtration to afford pure products in 57–98% yield.

Both elemental and spectral (^{1}H NMR and IR) analysis data of all the synthesized compounds are in full agreement with the suggested molecular structures. The IR spectra of pure products **5** {*1–35*} indicated the presence of broadened O–H and N–H bands related to hydroxyl and amide fragments, correspondingly, in the area of 3465–3011 cm^{-1}. Strong C=O bands at 1671–1719 cm^{-1} were also observed. The signals from C=N fragments at 1632–1656 cm^{-1} were less expressed and



4a: R^1 = H
4b: R^1 = 2-Me
4c: R^1 = 3-Me
4d: R^1 = 4-Me
4e: R^1 = 2-Et
4f: R^1 = 4-Et
4g: R^1 = 4-OMe
4h: R^1 = 2,4-di-OMe
4i: R^1 = 3,5-di-OMe
4j: R^1 = 2-F

Scheme 1. Synthesis of 2-arylimino derivatives of 2*H*-pyrano[2,3-*c*]pyridines. Reagents and conditions: (i) piperidine, MeOH, 40–45 °C, 20 min, yield 56–88%; (ii) AcOH, reflux, 20–30 min, yield 57–98%.

Table 1. 2*H*-Pyrano[2,3-*c*]pyridine derivatives synthesized in this work

Compound	R^1	R^2	R^3	Yield (%)
5{*1*}	H	H	4-COOEt	61
5{*2*}	H	H	H	83
5{*3*}	H	H	4-Et	64
5{*4*}	H	3,4-(–OCH_2O–)		93
5{*5*}	H	H	4-OPh	68
5{*6*}	H	H	4-MBT[a]	59
5{*7*}	H	3-Me	H	98
5{*8*}	H	3-Et	H	73
5{*9*}	H	3-OMe	5-OMe	80
5{*10*}	2-Me	3-Me	4-Me	91
5{*11*}	2-Me	3-Me	5-Me	78
5{*12*}	2-Me	3-Me	H	69
5{*13*}	3-Me	H	H	72
5{*14*}	3-Me	3-Me	4-Me	89
5{*15*}	3-Me	3-OMe	4-OMe	57
5{*16*}	3-Me	2-OMe	4-OMe	64
5{*17*}	3-Me	H	4-Me	76
5{*18*}	3-Me	H	4-OEt	77
5{*19*}	3-Me	3-OMe	5-OMe	83
5{*20*}	3-Me	3-Cl	H	61
5{*21*}	4-Me	3-Cl	4-Cl	67
5{*22*}	4-Me	2-OMe	H	91
5{*23*}	4-Me	H	4-OPh	88
5{*24*}	4-Me	3-OMe	H	85
5{*25*}	4-Me	2-I	H	74
5{*26*}	4-Me	3-Cl	H	72
5{*27*}	4-Me	H	4-Br	70
5{*28*}	4-Me	2-Et	H	81
5{*29*}	H	2-Ph	4-MBT[a]	71
5{*30*}	4-Et	3,4(–OCH_2O–)		72
5{*31*}	4-OMe	3-Cl	4-Cl	63
5{*32*}	3,5-DiOMe	H	4-$CONH_2$	65
5{*33*}	2,4-DiOMe	3-Cl	4-Cl	58
5{*34*}	2,4-DiOMe	3-OMe	4-OMe	74
5{*35*}	2-F	H	4-CF_3	60

[a] 4-MBT, 4-(6-methyl-1,3-benzothiazol-2-yl).

usually were overlapped with the signals from aromatic C=C bonds. ^{1}H NMR spectra of compounds **5** {*1–35*} showed characteristic signals from protons of the pyrano[2,3-*c*]pyridin-2-one heterocycle in the range of δ 8.50–8.67 (s, 1H, H-4) and δ 8.21–8.28 (s, 1H, H-6). All these spectra also contain resonances from methylene fragment (doublet at 4.69–4.74 ppm), hydroxyl (triplet at 5.37–5.44 ppm) and methyl group (singlet at 2.21–2.38 ppm). The protons of the amide group were observed as broad singlets at 7.71–7.95 and at 8.79–9.92 ppm.[14]

All the synthesized 2*H*-pyrano[2,3-*c*]pyridines **5** {*1–35*} were submitted for preliminary evaluation of their in vitro activity against *P. aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, and *Bacillis anthracoides*, and antimycotic activity against *Candida albicans*. The antimicrobial activity was determined by the double dilution method.[14,15] Suitable solvent control (DMF), positive growth control, and standard drug controls were also run simultaneously. Ciprofloxacin was chosen as a standard in antibacterial activity measurements, as it has excellent activity against most Gram-negative and Gram-positive bacteria, and is known as an important antibacterial drug in the treatment of a wide range of infections.[16]

Table 2 shows MIC values of the synthesized compounds. In general, the obtained compounds were very active in these in vitro experiments. As evident from the antibacterial screening data, 28 compounds (80% of the entire library) demonstrated significant activity against at least one strain of Gram-positive or Gram-negative microorganisms with MIC in the range of 12.5–25.0 μg/mL.

A total of 17 compounds were active against *B. anthracoides*; three of them displayed the same efficacy as the fluoroquinolone drug ciprofloxacin (MIC 12.5 μg/mL). The screening data revealed that except **5**{*12*}, **5**{*29*} and **5**{*31*} none of the obtained compounds were active against *P. vulgaris*. Eight compounds displayed the same efficacy as ciprofloxacin (MIC 12.5 μg/mL) in inhibition of *E. coli*.

Only three compounds, **5**{*14*}, **5**{*29*}, and **5**{*31*}, demonstrated significant activity against both Gram-positive and Gram-negative strains. Most of the synthesized compounds were active against *C. albicans*; 14 of them displayed even better efficacy than the standard antifungal drug, fluconazole. The most active antifungal compounds, **5**{*12*}, **5** {*19*}, **5**{*23*}, **5**{*30*}, and **5**{*31*}, displayed a 4-fold decrease in MIC (12.5 μg/mL) as

Table 2. Antimicrobal activity for 2*H*-pyrano[2,3-*c*]pyridine derivatives synthesized in this work

Compound	MIC, μg/mL					
	Sa	Ec	Pa	Pv	Ba	Ca
5{*1*}	50.0	50.0	50.0	50.0	50.0	50.0
5{*2*}	50.0	25.0	50.0	50.0	50.0	50.0
5{*3*}	50.0	50.0	50.0	50.0	25.0	50.0
5{*4*}	50.0	25.0	50.0	50.0	50.0	50.0
5{*5*}	50.0	25.0	50.0	50.0	50.0	50.0
5{*6*}	100.0	25.0	100.0	50.0	100.0	50.0
5{*7*}	100.0	100.0	100.0	100.0	100.0	100.0
5{*8*}	100.0	100.0	100.0	100.0	25.0	100.0
5{*9*}	50.0	50.0	25.0	50.0	50.0	100.0
5{*10*}	100.0	50.0	25.0	50.0	12.5	100.0
5{*11*}	100.0	100.0	50.0	100.0	100.0	100.0
5{*12*}	100.0	50.0	50.0	25.0	25.0	12.5
5{*13*}	100.0	50.0	25.0	50.0	25.0	12.5
5{*14*}	50.0	25.0	12.5	50.0	12.5	12.5
5{*15*}	50.0	50.0	100.0	100.0	50.0	50.0
5{*16*}	50.0	50.0	25.0	50.0	50.0	50.0
5{*17*}	50.0	50.0	50.0	50.0	50.0	50.0
5{*18*}	50.0	50.0	25.0	50.0	50.0	50.0
5{*19*}	25.0	100.0	50.0	100.0	50.0	12.5
5{*20*}	100.0	50.0	50.0	100.0	100.0	50.0
5{*21*}	100.0	100.0	50.0	50.0	25.0	25.0
5{*22*}	100.0	50.0	50.0	100.0	25.0	25.0
5{*23*}	50.0	50.0	50.0	50.0	50.0	12.5
5{*24*}	50.0	25.0	50.0	100.0	50.0	50.0
5{*25*}	25.0	50.0	50.0	100.0	50.0	50.0
5{*26*}	50.0	50.0	50.0	50.0	25.0	25.0
5{*27*}	25.0	50.0	50.0	50.0	25.0	25.0
5{*28*}	50.0	50.0	50.0	50.0	25.0	50.0
5{*29*}	50.0	25.0	50.0	25.0	12.5	25.0
5{*30*}	100.0	50.0	50.0	50.0	25.0	12.5
5{*31*}	12.5	25.0	25.0	25.0	25.0	12.5
5{*32*}	50.0	50.0	50.0	50.0	25.0	25.0
5{*33*}	50.0	50.0	50.0	50.0	25.0	50.0
5{*34*}	25.0	50.0	50.0	50.0	50.0	50.0
5{*35*}	50.0	50.0	50.0	100.0	25.0	25.0
Cipro	6.25	25.00	12.5	12.5	12.5	—
Fluc	—	—	—	—	—	50.0

Sa, *Staphylococcus aureus* (ATCC 25923); Ec, *Esherichia coli* (ATCC 25922); Pa, *Pseudomonas aeruginosa* (ATCC 27853); Pv, *Proteus vulgaris* (ATCC 4636); Ba, *Bacillis anthracoides* (ATCC 1312); Ca, *Candida albicans* (ATCC 885-653); Cipro, ciprofloxacin; Fluc, fluconazole; MIC, minimum inhibitory concentration. The tests were performed in triplicate.

compared to fluconazole. Seven compounds depicted in Table 2 showed inhibition at 25.0 μg/mL concentration. One compound, **5**{*31*}, displayed high activity (MIC 12.5–25 μg/mL) against all six pathogenic microorganisms studied in this work. Only five compounds synthesized in this work were inactive against the studied bacterial and fungal microorganisms.

Structure–activity relationships are not obvious for the synthesized pyrano[2,3-*c*]pyridines. Obviously, bulky substituents, such as phenyl, phenyloxy, and 1,3-benzothiazol-2-yl, at position 4 of the *N*-arylimino fragment are well tolerated among the active compounds. Moreover, compound **5**{*29*}, one of the most active antibacterial and antifungal agents in the studied series, has the largest *N*-arylimino group. As a rule, the introduction of halogen substituents in position 4 increased the activity.

In conclusion, a series of novel 5-hydroxymethyl-8-methyl-2-(*N*-arylimino)-2*H*-pyrano[2,3-*c*]pyridine-3-(*N*-aryl)-carboxamides was synthesized and characterized, and most of them proved to be potent antibacterial or antifungal agents. It was observed that the antimicrobial activity in this class of compounds is dependent on the nature of substituents at 2-(*N*-arylimino) and 3-(*N*-aryl)-carboxamide fragments. However, the structure–activity relationships are not clear yet for the synthesized molecules. The synthetic method we developed in this work is suitable for convenient preparation of large compound libraries and in-depth SAR exploration. Additional chemical sets around the active compound are being studied to identify compounds with improved in vitro antimicrobial activities, and those results will be reported in due course.

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11. Melting points were measured with a Buchi B-520 melting point apparatus and are uncorrected. IR spectra were recorded on Specord M80 spectrometers in KBr. ^{1}H NMR spectra were recorded on Varian Gemini-300 spectrometers in DMSO-d_6 using TMS as an internal standard. Chemical shifts were expressed in δ (ppm) relative to TMS as internal standard and coupling constants (J) in hertz. Elemental analyses were within ±0.4% of the theoretical values. General procedure for the synthesis of 2-imino-5-hydroxymethyl-8-methyl-2*H*-pyrano[2,3-*c*]pyridine-3-(*N*-aryl)carboxamides **4a**–**j**. A solution of 10.18 g (50 mmol) of pyridoxal hydrochloride **1** and 50 mmol of cyanoacetamide **2a**–**j** in 100 mL of absolute methanol was heated to 40–45 °C. Distilled piperidine (1.0 mL, 100 mmol) was slowly added, and the resulting mixture was allowed to stand at 40–45 °C for 20 min. The formed precipitate was filtered out, washed by methanol and recrystallized from ethanol to afford **4a**–**j** as yellow crystalline solid. Representative spectral data for compounds **4**. 2-Imino-5-hydroxymethyl-8-methyl-2*H*-pyrano[3,2-*c*]pyridin-3-(*N*-phenyl)carboxamide **4a**. Mp 230–231 °C; IR (v) 3300 (amid N–H), 3202 (imine N–H), 3000 (C–H), 1689 (C═O), 1634 (C═N), 1600 and 1560 (C═C), 1227 (C–O), 1009, 760; ^{1}H NMR (DMSO-d_6) δ 2.54 (3H, s, Me), 4.71 (2H, d, J = 8.2 Hz, CH_2), 5.41 (1H, t, J = 5.1 Hz, OH), 7.13 (1H, dt, J = 6.9 Hz, J = 1.6 Hz, ArH), 7.39 (2H, t, J = 7.8 Hz, ArH), 7.64 (2H, d, J = 9.4 Hz, ArH), 8.23 (1H, s, H-4), 8.61 (1H, s, H-6), 9.41 (1H, s, NH), 12.58 (1H, s, NH); Anal. Calcd for $C_{17}H_{15}N_3O_3$: C, 66.01; H, 4.89; N, 13.53. Found: C, 66.03; H, 4.89; N, 13.58. 2-Imino-5-hydroxymethyl-8-methyl-2*H*-pyrano[3,2-*c*]pyridin-3-[*N*-(2-methylphenyl)]-carboxamide **4b**. Mp 233–235 °C; IR (v) 3302 (amid N–H), 3211 (imine N–H), 3051 (C–H), 1690 (C═O), 1632 (C═N), 1592 and 1562 (C═C),1223 (C–O), 1011, 759; ^{1}H NMR (DMSO-d_6) δ 2.28 (3H, s, Me), 2.48 (3H, s, Me), 4.73 (2H, d, J = 5.1 Hz, CH_2), 5.37 (1H, t, J = 6.5 Hz, OH), 7.07 (1H, d, J = 7.5 Hz, ArH), 7.21 (2H, m, ArH), 8.17 (1H, d, J = 8.1 Hz, ArH), 8.24 (1H, s, H-4), 8.68 (1H, s, H-6), 9.45 (1H, s, NH), 12.38 (1H, s, NH); Anal. Calcd for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00. Found: C, 66.87; H, 5.29; N, 13.00.
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13. General procedure for the synthesis of 2-(*N*-aryl)-imino-5-hydroxymethyl-8-methyl-pyrano[3,2-*c*]pyridine-3-(*N*-aryl)carboxamides **5**{*1–35*}. To a solution of aromatic amine (1 mmol) in glacial acetic acid (5 mL) was added a solution of imine **4a**–**j** (1 mmol) in acetic acid, and the mixture was refluxed for 20–30 min. Then the reaction mixture was cooled, and the precipitated product was filtered and recrystallizated from EtOH, DMF, or EtOH–DMF. Structures and yields of **5**{*1-35*} are shown in Table 1. Representative spectral data for the active compounds **5**: 2-[*N*-(3,4-Dimethylphenyl)]-imino-5-hydroxymethyl-8-methyl-2*H*-pyrano[3,2-*c*]pyridin-3-[*N*-(3-methylphenyl)]-carboxamide (**5**{*14*}). Mp 268–269 °C; ^{1}H NMR (δ) 2.27 (s, 9H, Me), 2.40 (s, 3H, Me), 4.71 (d, 2H, J = 4 Hz, CH_2), 5.42 (t, 1H, J = 3.6 Hz, OH), 6.94 (d, 1H, J = 7.8 Hz, ArH), 7.18–7.29 (m, 4, ArH), 7.48 (m, 2H, ArH), 8.28 (s, 1H, H-6), 8.60 (s, 1H, H-4), 12.23 (s, 1H, NH). Anal. Calcd for $C_{26}H_{25}N_3O_3$: C, 73.05; H, 5.89; N, 9.83. Found: C, 73.05; H, 5.90; N, 9.80. 2-*N*-[4-(6-Methyl-1,3-benzothiazol-2-yl)phenyl]-imino-5hydroxymethyl-8-methyl-2*H*-pyrano[3,2-c]pyridin-3-[*N*-(2-ethylphenyl)] carboxamide (**5**{*29*}). Mp 273–274 °C; ^{1}H NMR (δ) 1.11 (t, 3H, J = 9.0 Hz, Et), 2.47 (s, 6H, Me), 2.70 (q, 2H, J = 10.3 Hz, Et), 4.75 (d, 2H, J = 5.2 Hz, CH_2), 5.49 (t, 1H, J = 3.9 Hz, OH), 7.10 (m, 1H, ArH), 7.21–7.35 (m, 3H, ArH), 7.52 (d, 2H, J = 9.4 Hz, ArH), 7.90 (d, 2H, J = 8.5 Hz, ArH), 8.13 (d, 2H, J = 9.4 Hz, ArH), 8.23 (d, 1, J = 8.6 Hz, ArH), 8.31 (s, 1H, H-6), 8.81 (s, 1H, H-4), 11.92 (s, 1H, NH). Anal. Calcd for $C_{33}H_{28}N_4O_3S$: C, 70.69; H, 5.03; N, 9.99; S, 5.72. Found: C, 70.70; H, 5.05; N, 10.00; S, 5.70. 2-[*N*-(3,4-Dichlorophenyl)]-imino-5-hydroxymethyl-8-methyl-2*H*-pyrano[3,2-*c*]pyridin-3-[*N*-(4-methoxyphenyl)]-carboxamide (**5**{*31*}). Mp 228 °C; ^{1}H NMR (δ) 2.32 (s, 3H, Me), 3.70 (s, 3H, OCH_3), 4.71 (d, 2H, J = 6.2 Hz, CH_2), 5.42 (t, 1H, J = 4.7 Hz, OH), 6.92 (d, 2H, J = 9.5 Hz, ArH), 7.36 (dd, J = 9.3 Hz, J = 0.9 Hz, 1H, ArH), 7.60 (d, J = 9.5 Hz, 2, ArH), 7.61 (d, J = 3.6 Hz, 1H, ArH), 7.75 (d, J = 0.9 Hz, 1H, ArH), 8.30 (s, 1H, H-6), 8.68 (s, 1H, H-4), 11.61 (s, 1H, NH). Anal. Calcd for $C_{24}H_{19}Cl_2N_3O_4$: C, 59.52; H, 3.95; N, 8.68. Found: C, 59.50; H, 3.96; N, 8.68.
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15. Experimental determination of antimicrobial activity. The bacterial strains were grown in Hottinger's broth (0.1% amine nitrogen and 0.5% NaCl, pH 7.0–7.2), and the fungal strain was grown in Sabouraud's dextrose broth. The tests were performed in assay tubes (12 × 75 mm) in triplicate. Ten tubes were filled with the seeded broth (2 mL). The test compound (2 mL of 1 mg/mL solution in DMF) was added to the first tube and 2 mL of this solution was transferred to the second tube and so on so forth. Then bacterial and fungal strains were inoculated in the tubes (bacteria 2×10^5 cfu/mL, fungi 5×10^5 cfu/mL). The tubes were incubated at 37 °C for bacteria (for 18–24 h) and 30 °C for fungi (for 48 h). The MICs (in μg/mL) were recorded by visual observation.
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