A Platinum Open Access Journal for Organic Chemistry

Paper

Free to Authors and Readers | DOAJ Seal | *Arkivoc* **2024** (8) 202412273

Synthesis, characterization and biological activity of some new dihydroisoquinolines and dihydrothieno [2,3-*c*]isoquinolines

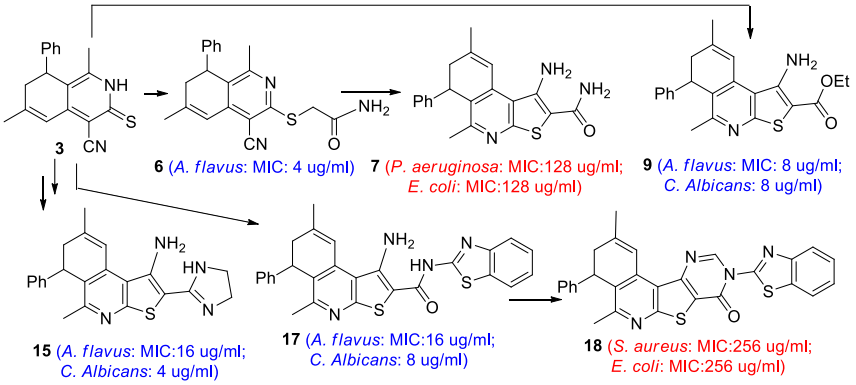
Etify A. Bakhite,[a]* Omaima F. Ibrahim,[a] Esraa Khamies,[a] Shams H. Abdel-Hafez,[b] Maha Q. M. Qahtan[c] and Islam S. Marae[a]

[a]Chemistry Department, Faculty of science, Assiut University, Assiut 71516, Egypt
[b]Department of Chemistry, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia.
[c]Department of Chemistry, Faculty of Science, Taiz University, Taiz, Yemen.
Email: etafy@aun.edu.eg

Received mm-dd-yyyy **Accepted** mm-dd-yyyy **Published on line** mm-dd-yyyy

Abstract

In this work, a versatile compound, 4-cyano-1,6-dimethyl-8-phenyl-7,8-dihydroisoquinoline-3(2*H*)-thione (**3**) was synthesized and utilized as a starting compound for the preparaton of the title compounds. Thus, reaction of **3** with iodomethane, 2-chloroacetamide, ethyl chloroacetate, chloroacetonitrile or *N*-(benzthiazol-2-yl)-2-chloroacetamide by heating in ethanol containing sodium acetate, gave 3-unsubstituted or 3-substituted methylsulfanyl-7,8-dihydroisoquinoline-4-carbonitriles **4** or **6**, **8**, **10** and **16** respectively. The latter compounds **(6**, **8**, **10** and **16**) contain an active methylene group that adds easily to the carbonitrile group to build a thiophene ring, fused to an isoquinoline moiety, upon heating with sodium ethoxide in ethanol thus affording the corresponding 6,7-dihydrothieno[2,3-*c*]isoquinolines **7**, **9**, **11** and **17**, in nearly quantitative yields. Compounds **9**, **11** and **17** underwent further reactions with some reagents to give other 6,7-dihydrothieno[2,3-*c*]isoquinolines **12-15** and 3,4-dihydropyrimidothienoisoquinoline **18**. All synthesized compounds were screened for their biological activity as bactericidal and fungicidal agents. Some of them showed promising antifungal activity.



Keywords: Dihydroisoquinolines, dihydrothieno[2,3-*c*]isoquinolines.
Cite as *Arkivoc* **2024** (8) 202412273
DOI: https://doi.org/10.24820/ark.5550190.p012.273

Introduction

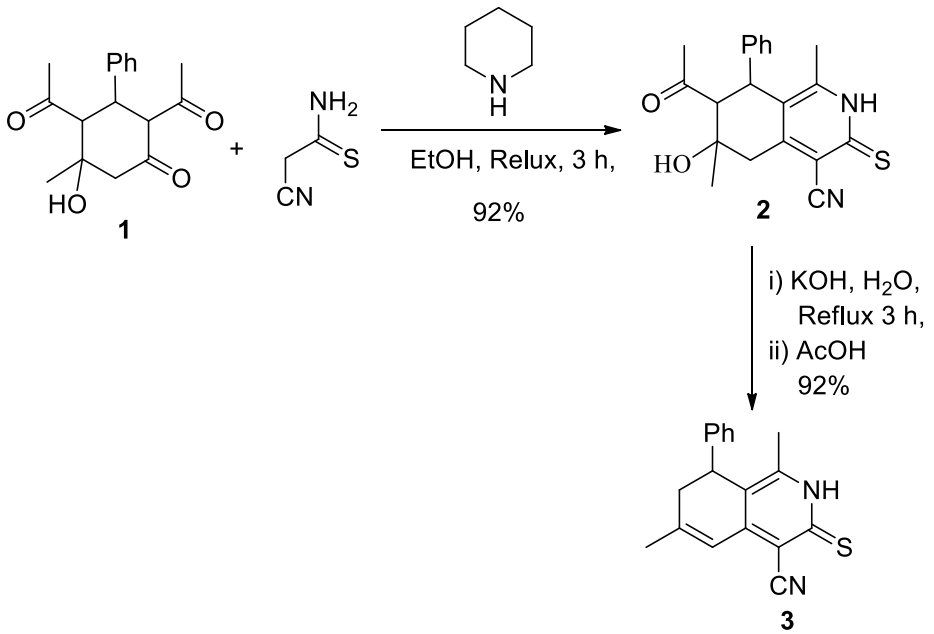
Isoquinolines are heterocyclic aromatic compounds that possess various pharmacological functions, including anti-HIV,[1] antitumor, antifungal,[4,5] antibacterial,[5-9] antioxidant,[10] anti-inflammatory,[10,11] and insecticidal activities.[12] Isoquinoline derivatives are used as anesthetics, antihypertension agents, disinfectants, and vasodilators.[9]

Dihydroisoquinolines (DHISQs) are important class of heterocyclic compounds. They have good medicinal and pharmaceutical applications where they used as JNK3, PRMT5 inhibitors, human cytomegalovirus inhibitors and enhancers for EZH2 inhibitors.[13-15] They also have antimicrobial and antifungal activity.[16-18] There are many isomers of the DHISQs with the 7,8-DHISQs being less common because their syntheses are difficult. Synthesis of 7,8-DHISQs by using the 5,6,7,8-THISQ derivatives as precursors,[19,20] or extraction from metabolites of isoquinolines, have been reported.[21,22] A literature survey revealed that there is a little work on the chemistry and applications of 7,8-dihydroisoquinolines despite the medicinal and biological importance of such compounds. Some compounds related to 7,8-DHISQs were used as intermediates for synthesis of mimosamycin,[23] potent inhibitors of Lck [24] and AMV reverse transcriptase.[25] Certain 7,8-DHISQs were reported to have anticancer activity.[26] In our previous work, we have decribed the synthesis, characterization and applications of various poly-substituted 5,6,7,8-tetrahydroisoquinolines and 7,8-DHISQs.[27-34] In this paper, we describe further synthesis, reactions, characterization and crystal structure of new 7,8-DHISQs in order with the hope that they may have good biological and medicinal applications. All synthesized compounds were screened for their biological activity as bactericidal and fungicidal agents.

Results and Discussion

Synthesis and characterization

Our approach to the synthesis of the target compounds starts from 4-cyano-1,6-dimethyl-8-phenyl-7,8-dihydroisoquinoline-3(2*H*)-thione (**3**) which was prepared according to our reported method (Scheme 1).[35]

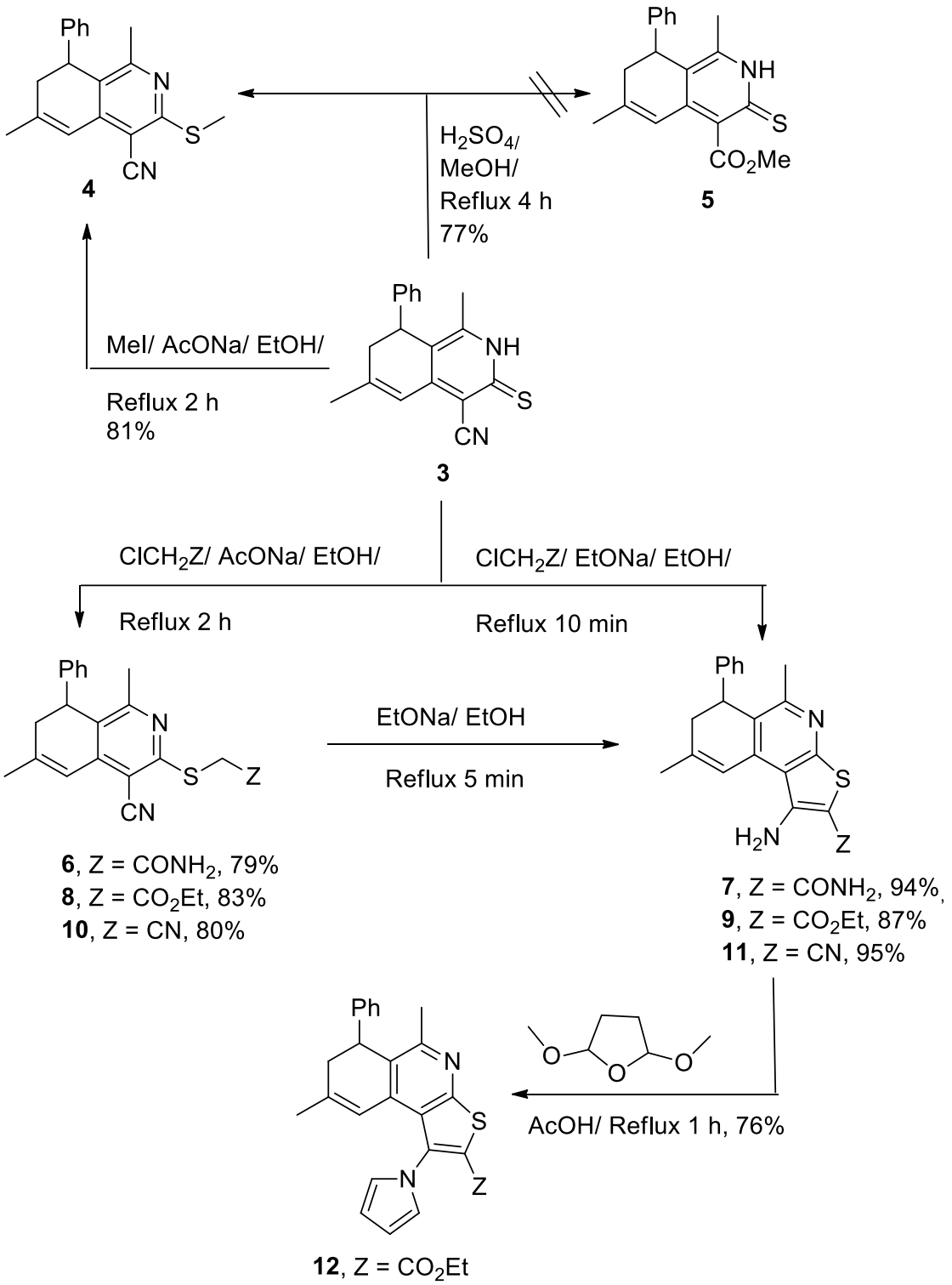


Scheme 1 Synthesis of 4-cyano-1,6-dimethyl-8-phenyl-7,8-dihydroisoquinoline-3(2*H*)-thione **(3)**

The IR spectrum of **3** revealed the absence of both hydroxyl and acetyl groups of compound **2**. The NMR spectra confirmed the absence of each hydroxyl, acetyl and methylene group at position 5 and the presence of s methine group at position 5 and methylene group at position 7. The structure of **3** was further confirmed and supported *via* determination of the crystal structure of its *S*-ethylsulfanyl derivative.[35]

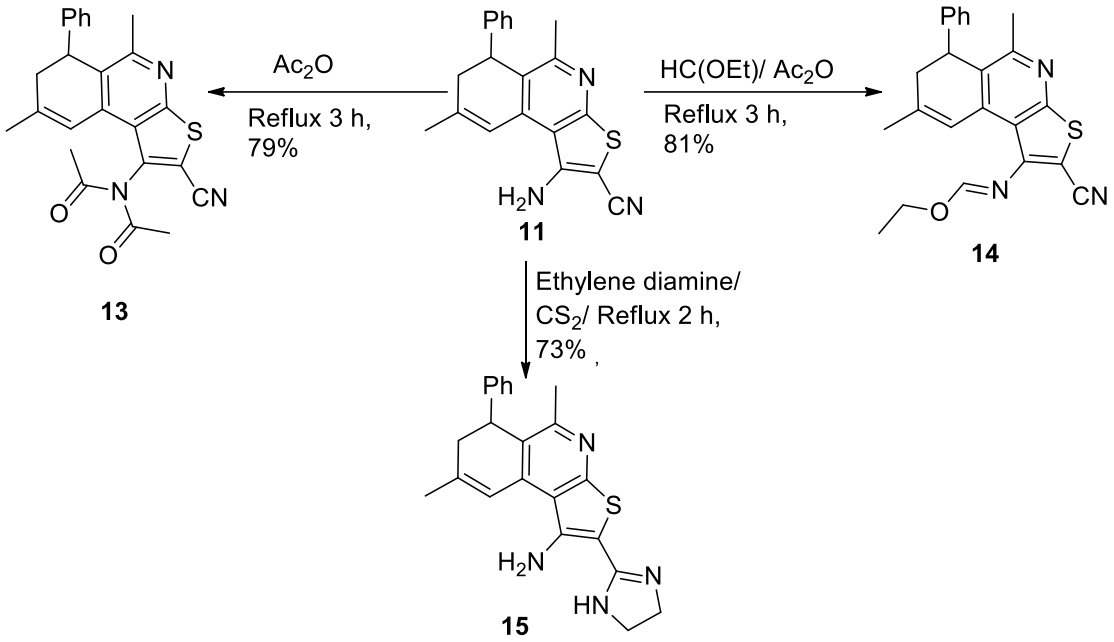
The chemistry of the 7,8-dihydroisoquinoline **3** was explored by carrying out some reactions on the thione and carbonitrile groups. An attempt to convert the carbonitrile group of compound **3** into methyl ester by refluxing in methanol containing catalytic amounts of concentrated H_2SO_4 failed and instead, the *S*-methylated derivative **4** was obtained (Scheme 2). The elemental and spectral analyses indicated that the product of the latter reaction possesses structure **4** rather than **5**. This ssurprising result was confirmed *via* an independent synthesis of **4**, by heating compound **3** with iodomethane in ethanol in the presence of sodium acetate.

Although the desired product **5** was not obtained, the latter reaction provided a good eco-friendly method for methylation of the thiol group. Also, the sulfur atom of the compound **3** was alkylated successfully with other simple alkylating agents (Scheme 2), namely : 2-chloroacetamide, ethyl chloroacetate, chloroacetonitrile or *N*-(benzthiazol-2-yl)-2-chloroacetamide, by heating in ethanol containing sodium acetate to give 3-*S*-(substituted)methylsulfanyl-7,8-dihydroisoquinoline-4-carbonitriles **6**, **8**, **10** or **16**, respectively. Each of compounds **6**, **8**, **10** and **16** contains active methylene group that added easily to the carbonitrile group to build a thiophene ring fused with an isoquinoline moiety, upon heating with sodium ethoxide in ethanol producing the corresponding 6,7-dihydrothieno[2,3-*c*]isoquinolines **7**, **9**, **11** and **17** in nearly quantitative yields. The latter compounds were also prepared *via* direct reaction of compound **3** with the approipirate halocompounds in boiling ethanol in the presence of an excess amount of sodium ethoxide. Despite the high molecular weight of compound **9**, its amino group is still active and was reacted successfully with 2,5-dimethoxytetrahydrofuran to build a pyrrole ring and thus compound **12** was obtained (Scheme 2**)**.



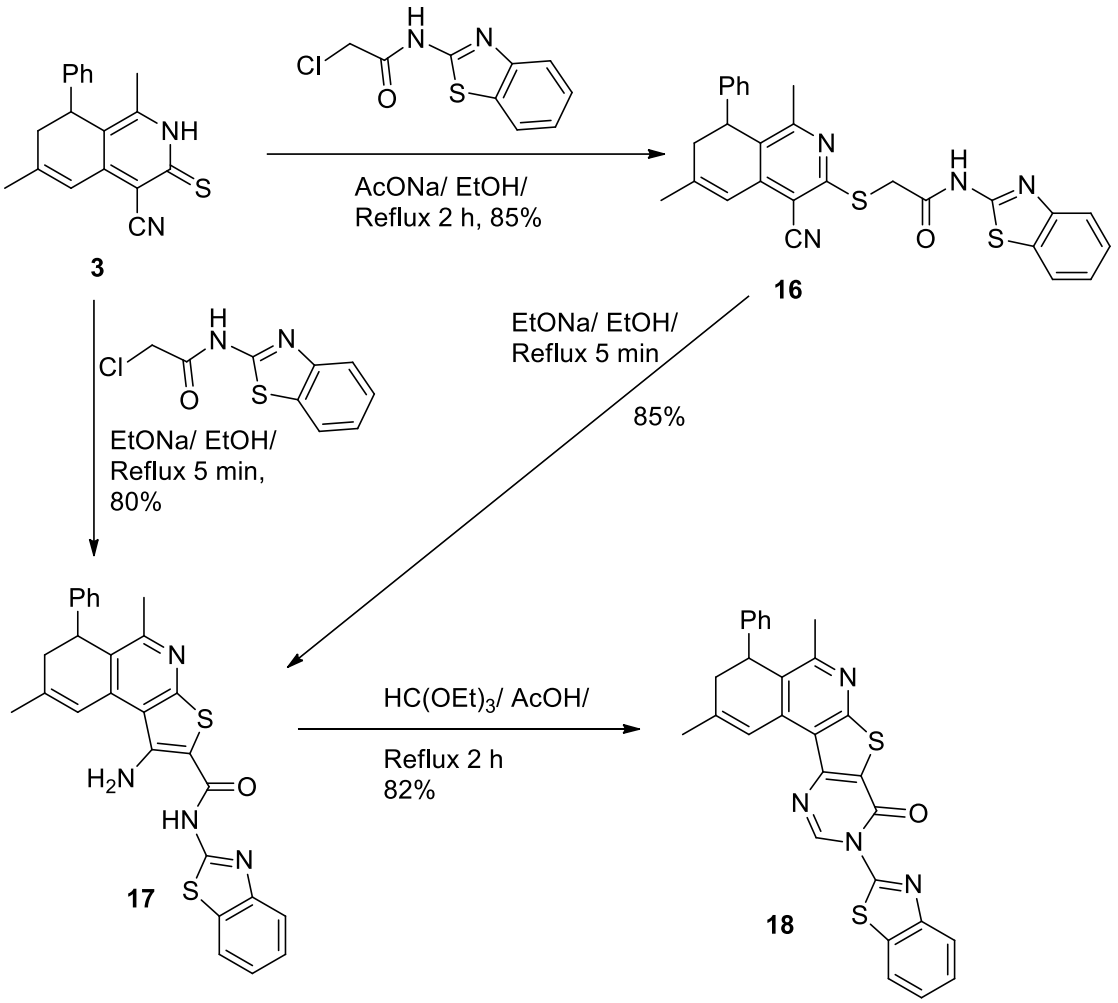
Scheme 2. Synthesis of compounds **4** and **6-12**

Compound **11** was utilized as a key intermediate for preparing other new 6,7-dihydrothieno[2,3-*c*]isoquinoline derivatives. Thus, heating of **11** with an excess of acetic anhydride furnished diacetyl derivative **13** in excellent yield. Also, it condensed with triethyl orthoformate in the presence of acetic anhydride to afford formimidate compound **14**. An attempt to convert the carbonitrile group of **11** into a dihydroimidazole ring *via* reaction with ethylenediamine in the presence of carbon disulfide as a catalyst was successful and thus imidazolylthienoisoquinoline **15** was obtained (Scheme 3).



Scheme 3. Synthesis of 6,7-dihydrothieno[2,3-*c*]isoquinolines **13-15**

In an attempt to join the 7,8-DHISQ framework with other important heterocyclic moieties such as benzthiazole was carried out *via* reaction of **3** with *N*-(benzthiazol-2-yl)-2-chloroacetamide producing benzthiazolyl derivative **16**. Cyclization of **16** into its isomeric compound, 1-amino-2-(benzthiazol-2-yl)-6,7-dihydrothieno[2,3-*c*]isoquinoline **17** was carried by heating with a catalytic amount of sodium ethoxide in ethanol. Compound **17** was also obtained by direct reaction of **3** with *N*-(benzthiazol-2-yl)-2-chloroacetamide in the presence of an excess amount of sodium ethoxide. Both amino and amido groups of compound **17** condensed with triethyl orthoformate in boiling glacial acetic acid to furnish 3,4-dihydropyrimidothienoisoquinoline **18** (Scheme 4).



Scheme 4 Synthesis of 6,7-dihydrothieno[2,3-c]isoquinolines **16-18**

Characterization

Structures of all newly synthesized compounds were characterized by elemental and spectral analyses. (*cf.* Experimental Section)

Biological activity

All synthesized compounds were evaluated as bactericidal agents towards four strains of bacteria (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*) and as antifungals towards two fungal species (*Aspergillus flavus* and *Candida Albicanss*) following the dilution method.[36] The results obtained are expressed as Minimal Inhibition Concentration (MIC) (ug/ml) and are given in Table 1. These results revealed that: (i) most compounds showed inhibition effects ranged from weak to moderate towards all bacterial strains under investigation; (ii) only compounds **7** and **13** showed moderate activities against Gram -ve bacteria, while others are active in the top testing concentration of 256 ug/ml; (iii) only two compounds **9** and **17** which showed no activity against all bacterial strains; (iii) most compounds exhibited antifungal activity ranged from weak to strong towards the two fungal species: (v) only two compounds **8** and **13** showed no antifungal activity and (vi) a number of compounds **4**, **6**, **9**, **15** and **17** showed high antifungal activity near to that of the reference.

Table 1. Minimal Inhibition Concentration (MIC) of all synthesized compounds (ug/ml)

Compd. No.	Gram +ve bacteria[a]		Gram –ve bacteria[b]		*A. flavus*[c]	***C.** Albicans*[c]
	***S.** aureus*	*S. pneumoniae*	P. aeruginosa	E. Coli		
4	-	-	256	-	8	-
6	256	256	-	256	4	256
7	256	256	128	128	256	128
8	256	-	-	-	-	-
9	-	-	-	-	8	8
10	-	256	-	-	128	-
11	256	-	-	-	128	-
12	-	256	256	-	-	256
13	256	256	128	256	-	-
14	256	256	-	-	128	256
15	256	256	256	256	16	4
16	-	-	256	-	64	-
17	-	-	-	-	16	8
18	256	-	-	256	128	256

[a] Reference of Gram negative bacteria --------- Colistin: 1 ug/ml

[b] Reference of Gram positive bacteria----------Ciprofloxacin : 4 ug/ml

[c] Reference of *Aspergillus flavus* & *Candida Albicans* -------- Linezolid : 4 ug/ml

Conclusions

In this paper, we have reported the successful synthesis and characterization of new sixteen dihydroisoquinoline derivatives with biological and medicinal applications, in very pure state and high yields. These derivatives comprise four categories: (i) methylsulfanyl-7,8-dihydroisoquinoline-4-carbonitrile **4**; (ii) functionally 3-*S*-substituted methylsulfanyl-7,8-dihydroisoquinoline-4-carbonitriles **6**, **8**, **10** and **16**; (iii) 1-amino-2-substituted 6,7-dihydrothieno[2,3-*c*]isoquinolines **7**, **9**, **11**, **17** and (iv) 3,4-dihydropyrimido [4',5':4,5]thieno [2,3-*c*]isoquinoline-8(9*H*)-one derivative **18**. Furthermore, all synthesized compounds were evaluated for their antibacterial and antifungal activity and some of them showed promising activity as fungicidal agents.

Experimental Section

General. IR spectra: Shimadzu 470 IR-spectrophotometer (KBr; ν_{max} in cm^{-1}); ^1H NMR spectra: Brucker 400 MHz spectrometer using $CDCl_3$ as a solvent (except for compounds **2b** and **15** which were dissolved in DMSO-d_6) and tetramethylsilane as internal reference (chemical shifts are given in δ; ppm and J values are given in Hertz; Hz); elemental analyses: Perkin Elmer 2400 LS Series CHN/O analyser.

4-Cyano-7,8-dihydro-1,6-dimethyl-8-phenylisoquinoline-3(2*H*)-thione (3) was synthesised from compound **2** following our previous method.[35]

Action of H_2SO_4/MeOH mixture on compound 3. To a suspension of **3** (1.46 g, 0.005 mol) in MeOH (50 mL), analar H_2SO_4 (4 mL) was added dropwise with stirring. The mixture was heated at reflux for 4 h., alowed to cool and neutralized with an aqueous solution of Na_2CO_3 (10%) whereby a white substance formed. It was collected, dried in air and recrystallized from MeOH to give colorless needles. This compound was identified as **4-cyano-7,8-dihydro-1,6-dimethyl-3-methylsulfanyl-8-phenylisoquinoline** (**4**). Yield: 77%; mp 90-92 °C. IR: 3084 (sp^2 CH); 2962, 2927 (sp^3 CH); 2212 (C≡N); 1636 (C=N). ^{1}H NMR: δ 7.19-7.23 (m, 3H, ArH); 6.98 (d, *J* 8, 2H, ArH); 6.60 (s, 1H, C^5H); 4.18 (d, *J* 8, 2H, C^8H); 2.93-2.98 (dd, J_1,J_2 = 8, 1H, C^7H); 2.59 (s, 3H, SCH_3); 2.49 (d, *J* 16, 1H, C^7H); 2.31 (s, 3H, CH_3); 1.86 (s, 3H, CH_3). Anal. Calcd. For $C_{19}H_{18}N_2S$ (306.12): C, 74.47; H, 5.92; N, 9.14%. Found: C, 74.31; H, 6.17; N, 9.23%.

Reaction of compound 3 with iodomethane, 2-chloroacetamide, ethyl chloroacetate, chloroacetonitrile or *N*-(benzthiazol-2-yl)-2-chloroacetamide; formation of compounds 4, 6, 8, 10 and 16. General procedure

A mixture of compound **3** (2.92 g, 0.01 mol), appropriate halocompound (0.01 mol) and AcONa.$3H_2O$ (1.50 g, 0.011 mol) was suspended in EtOH (100 mL) and then hated at reflux for 2 h. During reaction time, the yellow colour disappeared gradually to reach a colorless solution. The colorless crystalline compound that precipitated was collected and recrystallized from ethanol to give **4**, **6**, **8**, **10** or **16**.

4-Cyano-7,8-dihydro-1,6-dimethyl-3-methylsulfanyl-8-phenylisoquinoline (4). The compound was synthesized by reaction of **3** with iodomethane (yield: 81%) in analogy to the reported method.[35]

(4-Cyano-7,8-dihydro-1,6-dimethyl-8-phenylisoquinolin-3-ylsulfanyl)acetamide (6). This compound was synthesized by using 2-chloroacetamide. Yield: 79%; m.p. 166-168 °C. IR: 3442, 3313, 3183 (NH_2); 2932 (sp^3 CH); 2218 (C≡N); 1673 (C=O). ^{1}H NMR: 7.20-7.23 (m, 3H, ArH); 6.98 (d, *J* 8, 2H, ArH); 6.61 (s, 1H, C^5H); 5.76 (s, 2H, NH_2); 4.18 (d, *J* 8, 2H, C^8H); 3.79-3.89 (dd, J_1,J_2 = 12, 2H, SCH_2); 2.93-2.98 (dd, J_1,J_2 = 8, 1H, C^7H); 2.52 (d, *J* 16, 1H, C^7H); 2.31 (s, 3H, CH_3); 1.88 (s, 3H, CH_3). Anal. Calcd. For $C_{20}H_{19}N_3OS$ (349.12): C, 68.74; H, 5.47; N, 12.02%. Found: C, 69.14; H, 5.52; N, 11.87%.

Ethyl (4-cyano-7,8-dihydro-1,6-dimethyl-8-phenylisoquinolin-3-ylsulfanyl)acetate (8). This compound was synthesized by using ethyl chloroacetate. Yield: 83%; m.p. 122-124 °C. IR: 3074, 3035 (sp^2 CH); 2977, 2920 (sp^3 CH); 2212 (C≡N); 1736 (C=O); 1650 (C=N). ^{1}H NMR: 7.19-7.22 (m, 3H, ArH); 6.97 (d, *J* 8, 2H, ArH); 6.59 (s, 1H, C^5H); 4.14-4.20 (m, 3H: C^8H and OCH_2); 3.91-3.99 (dd, J_1,J_2 = 12, 2H, SCH_2); 2.91-2.96 (dd, J_1,J_2 = 8, 1H, C^7H); 2.49 (d, *J* 16, 1H, C^7H); 2.25 (s, 3H, CH_3); 1.86 (s, 3H, CH_3); 1.22-1.25 (t, *J* 6, 3H, OCH_2CH_3). Anal. Calcd. For $C_{22}H_{22}N_2O_2S$ (378.14): C, 69.81; H, 5.86; N, 7.40 %. Found: C, 69.58; H, 6.01; N, 7.09%.

4-Cyano-7,8-dihydro-1,6-dimethyl-8-phenylisoquinolin-3-ylsulfanyl)acetonitrile (10). This commpound was synthesized by using chloroacetonitrile. Yield: 80%; m.p. 125-127 °C. IR: 2247 (C≡N, non conjugated); 2223 (C≡N, conjugated); ^{1}H NMR: 7.22-7.24 (m, 3H, ArH); 7.99 (d, *J* 8, 2H, ArH); 6.61 (s, 1H, C^5H); 4.21 (d, *J* 8, C^8H); 3.97-4.07 (dd, J_1,J_2 = 12, 2H, SCH_2); 2.95-3.00 (dd, J_1,J_2 = 12, 1H, C^7H); 2.53 (d, *J* 8, 1H, C^7H), 2.36 (s, 3H, CH_3); 1.89 (s, 3H, CH_3). Anal. Calcd. For $C_{20}H_{17}N_3S$ (333.11): C, 72.48; H, 5.17; N, 12.68 %. Found: C, 72. 66; H, 5.14; N, 13.01 %.

3-(*N*-(Benzthiazol-2-yl)carbamoylmethylsulanyl)-4-cyano-7,8-dihydro-1,6-dimethyl-8-phenylisoquinoline (16). This compound was synthesized by using *N*-(benzthiazol-2-yl)-2-chloroacetamide. Yield: 85%; m.p. 186-188 °C. IR: 3106 (sp^2 CH); 2972, 2925 (sp^3 CH); 2222 (C≡N); 1690 (C=O). 1H NMR: 7.03-7.50 (m, 10H: NH and ArH), 6.65 (s, 1H, C^5H); 4.07-4.24 (m, 3H: SCH_2 and C^8H); 2.96-3.03 (dd, J_1,J_2 = 8, 1H, C^7H); 2.54 (d, *J* 16, 1H, C^7H), 2.43 (s, 3H, CH_3); 1.91 (s, 3H, CH_3). Anal. Calcd. For $C_{27}H_{22}N_4OS_2$ (482.12): C, 67.20; H, 4.59; N, 11.61%. Found: C, 66.87; H, 4.63; N, 11.29 %.

Synthesis of 2-functionalized 1-amino-6,7-dihydro-5,8-dimethyl-6-phenylthieno[2,3-*c*]isoquinolines 7, **9**, **11 and 17. General methods**

Method A. To a solution of compound **6**, **8**, **10** or **16** (0.005 mol) in abs. EtOH (25 mL); ethanolic EtONa solution (0.5 mL, 5 %) was added. The resulting mixture was heated for 5 mins whereby a yellow precipitate formed. It was collected and recrystallized from EtOH to give **7**, **9**, **11** or **17.**

1-Amino-2-carbamoyl-6,7-dihydro-5,8-dimethyl-6-phenylthieno[2,3-c]isoquinoline (7). Thhis compound was synthesized from compound **6**. Yield: 94%; m.p. 272-274 °C. IR: 3497, 3449, 3307, 3264, 3148 (2 NH_2); 2974, 2933 (sp^3 CH); 1660 (C=O). 1H NMR: 7.19-7.25 (m, 3H, ArH); 6.97-7.04 (m, 3H: C^9H and ArH); 6.34 (s, 2H, $CONH_2$); 5.39 (s, 2H, NH_2); 4.27 (d, *J* 6, 1H, C^6H); 2.95-2.98 (dd, J_1,J_2 = 6, 1H, C^7H); 2.51 (d, *J* 12, 1H, C^7H); 2.38 (s, 3H, CH_3); 1.87 (s, 3H, CH_3). Anal. Calcd. For $C_{20}H_{19}N_3OS$ (349.12): C, 68.74; H, 5.47; N, 12.02%. Found: C, 68.51; H, 5.61; N, 12.18%.

Ethyl 1-amino-6,7-dihydro-5,8-dimethyl-6-phenylthieno[2,3-c]isoquinoline-2-carboxylate (9) was synthesized from compound **8**. yield: 87%; mp 177-180 °C. IR: 3494, 3343 (NH_2); 2970, 2928 (sp^3 CH); 1667 (C=O). ^1H NMR: δ 7.16-7.20 (m, 3H, ArH); 6.96-7.00 (m, 3H: C^9H and ArH); 6.11 (broad s, 2H, NH_2); 4.33-4.37 (q, *J* 8, 2H, OCH_2); 4.25 (d, *J* 8, 1H, C^6H); 2.93-2.97 (dd, J_1,J_2 = 6, 1H, C^7H); 2.49 (d, *J* 12, 1H, C^7H); 2.37 (s, 3H, CH_3); 1.86 (s, 3H, CH_3); 1.37-1.40 (t, *J* 6, 3H, $OCH_2\underline{CH_3}$). Anal. Calcd. For $C_{22}H_{22}N_2O_2S$ (378.14): C, 69.81; H, 5.86; N, 7.40%. Found: C, 70.13; H, 6.08; N, 7.68%.

1-Amino-2-cyano-6,7-dihydro-5,8-dimethyl-6-phenylthieno[2,3-*c*]isoquinoline (11). It was synthesized from compound **10**. Yield: 95%; mp 142-144 °C. IR: 3399, 3328, 3238 (NH_2); 3098 (sp^2 CH), 2987, 2925 (sp^3 CH); 2190 (C≡N). ^1H NMR: 7.13-7.20 (m: 4H: C^9H and ArH); 6.97-6.98 (d, 2H, ArH); 6.60 (s, 2H, NH_2); 4.35 (d, *J* 8, C^6H); 2.88-2.93 (dd, J_1,J_2 = 8, 1H, C^7H); 2.42 (d, *J* 16, 1H, C^7H), 2.26 (s, 3H, CH_3); 1.81 (s, 3H, CH_3 at C-8). Anal. Calcd. For $C_{20}H_{17}N_3S$ (333.11): C, 72.48; H, 5.17; N, 12.68%. Found: C, 72. 26; H, 5.13; N, 13.05%.

1-amino-2-(*N*-(benzthiazol-2-yl)carbamoyl)-6,7-dihydro-5,8-dimethyl-6-phenylthieno[2,3-c]isoquinoline (17). It was synthesized from compound **16**. Yield: 96%; mp 189-191 °C. IR: 3453, 3314, 3150 (NH_2, NH); 2987, 2915 (sp^3 CH); 1645 (C=O). ^1H NMR: 13.26 (s, 1H, NH); 6.98-7.84 (m, 12H: NH_2, C^9H and ArH), 4.34 (d, *J* 8, C^6H); 2.90-2.93 (dd, J_1,J_2 = 6, 1H, C^7H); 2.45 (d, *J* 12, 1H, C^7H), 2.26 (s, 3H, CH_3); 1.83 (s, 3H, CH_3). Anal. Calcd. For $C_{27}H_{22}N_4OS_2$ (482.12): C, 67.20; H, 4.59; N, 11.61%. Found: C, 66.91; H, 4.70; N, 11.99 %.

Method B. To a mixture of **3** (1.46 g, 0.005 mol) and 2-chloroacetamide, ethyl chloroacetate, chloroacetonitrile or *N*-(benzthiazol-2-yl)-2-chloroacetamide (0.005 mol) in abs. EtOH (30 mL), an ethanolic EtONa solution (10 mL, 5%) was added. The resulting mixture was refluxted for 10 mins. The crystalline yellow solid that was obtained was identified as compound **7**, **9**, **11** or **17** respectively; yields: 78-81%.

Ethyl 1-(pyrrol-1-yl)-6,7-dihydro-5,8-dimethyl-6-phenylthieno[2,3-*c*]isoquinoline-2-carboxylate (12). A mixture of **9** (1.89 g, 0.005 mol), 2,5-dimethoxytetrahydrofuran (1.0 mL) in glacial acetic acid (20 mL) was heaed at reflux for one hour. The formed precipitate was collected and crystallized from EtOH to give colourless crystals of **12**. Yield: 76%; mp 148-150 °C. IR: 3098 (sp^2 CH), 2987, 2925 (sp^3 CH); 1698 (C=O). ^1H NMR: 7.16-7.19 (m, 3H, ArH); 6.96 (d, *J* 8, 2H, ArH); 6.88 (s, 1H, pyrryl-H); 6.69 (s, 1H, pyrryl-H); 6.40 (d, *J* 16, 2H, pyrryl-H); 5.03 (s, 1H, C^9H); 4.23-4.27 (m, 3H: C^6H and OCH_2); 2.89 (dd, J_1,J_2 = 8, 1H, C^7H); 2.43 (d, *J* 16, 1H, C^7H); 2.45 (s, 3H, CH_3); 1.59 (s, 3H, CH_3); 1.22-1.25 (t, *J* 6, 3H, CH_3 of ester). Anal. Calcd. For $C_{26}H_{24}N_2O_2S$ (428.16): C, 72.87; H, 5.64; N, 6.54%. Found: C, 73.07; H, 5.60; N, 6.38 %.

2-Cyano-1-(*N*,*N*-diacetylamino)-6,7-dihydro-5,8-dimethyl-6-phenylthieno[2,3-*c*]isoquinoline (13). A solution of **11** (1.66 g, 0.005 mol) in redistilled Ac_2O (20 mL) washeated at reflux for 3 h., diluted with H_2O (30 mL). The solid that formed was collected, dried in air and crystallized from methanol to give colorless needles of **13**. Yield: 79%; mp 249-251 °C. IR: 3078 (sp^2 CH); 2972, 2924 (sp^3 CH); 2217 (C≡N); 1722 (C=O); 1645 (C=N).

1HNMR: 7.23-7.28 (m, 3H, ArH); 6.99 (d, *J* 4, 2H, ArH); 6.55 (s, 1H, C^9H); 4.33 (d, *J* 8, 1H, C^6H); 2.97-3.04 (dd, J_1,J_2 = 8, 1H, C^7H); 2.56 (d, *J* 16, 1H, C^7H), 2.52 (s, 3H, $COCH_3$); 2.47 (s, 3H, $COCH_3$); 2.34 (s, 3H, CH_3); 1.86 (s, 3H, CH_3). Anal. Calcd. For $C_{24}H_{21}N_3O_2S$ (415.14): C, 69.37; H, 5.10; N, 10.12%. Found: C, 69.54; H, 5.01; N, 9.84%.

Ethyl-*N*-(2-cyano-6,7-dihydro-5,8-dimethyl-6-phenylthieno[2,3-c]isoquinolin-1-yl)formimidate (14). A mixture of **11** (1.66 g, 0.005 mol), $HC(OEt)_3$ (1.0 mL) in redistilled Ac_2O (10 mL) was refluxed for 3 h. and then left to cool. The precipitate that formed was collected and crystallized from methanol to afford compound **14** in the form of colorless plates. Yield: 81%; mp 159-160 ºC. IR: 3082, 3027 (sp^2 CH), 2977, 2934 (sp^3 CH); 2207 (C≡N); 1630 (C=N). ^1H NMR: δ 8.05 (s, 1H, N=CH); 7.44 (s, 1H, C^9H); 7.16-7.25 (m, 3H, ArH); 6.95-7.01 (m, 2H, ArH); 4.49-4.54 (q, *J* 6, 2H, OCH_2); 4.28 (d, J 8, C^6H); 2.92-2.98 (dd, J_1,J_2 = 8, 1H, C^7H); 2.41 (d, J 16, 1H, C^7H), 2.40 (s, 3H, CH_3); 1.84 (s, 3H, CH_3); 1.46-1.50 (t, J 8, 3H, $OCH_2\underline{CH_3}$). Anal. Calcd. For $C_{24}H_{23}N_3OS$ (401.16): C, 71.79; H, 5.77; N, 10.47%. Found: C, 71.42; H, 5.60; N, 10.77%.

1-Amino-6,7-dihydro-2-(4,5-dihydro-1*H*-imidazol-2-yl)-5,8-dimethyl-6-phenylthieno[2,3-c]isoquinoline (15). A mixture of **11** (1.66 g, 0.005 mol), ethylenediamine (3 mL) and carbon disulphide (1.0 mL) was refluxed for 2 h, triturated with ethanol and then left to cool. The formed solid was collected, dried in air and recrystalized from ethanol to give yellow needles of **15**. Yield: 73%; mp 216-218 °C. IR: 3441, 3181 (NH_2, NH); 2918, 2864 (sp^3 CH); 1649 (C=N); 1602, 1582 (C=C). ^1H NMR: 6.98-7.02 (m, 8H: NH_2, C^9H and ArH); 6.56 (broad s, 1H, NH); 4.33 (d, *J* 8, 1H, C^6H); 3.57 (broad s, 4H, CH_2CH_2); 2.88-2.93 (dd, J_1,J_2, 1H, C^7H), 2.41 (d, *J* 16, 1H, C^7H), 2.26 (s, 3H, CH_3); 1.81 (s, 3H, CH_3). Anal. Calcd. For $C_{22}H_{22}N_4S$ (374.16): C: 70.56; H, 5. 92; N, 14.96 %. Found: C: 70.50; H, 5. 71; N, 15.07 %.

9-(Benzthiazol-2-yl)-3,4-dihydro-2,5-dimethyl-4-phenylpyrimido[4',5':4,5]thieno[2,3-*c*]isoquinoline-8(9*H*)-one (18). A mixture of **17** (2.41g, 0.005 mol) and $HC(OEt)_3$ (1.0 mL) in glacial AcOH (20 mL) was heated at refluxefor 2 h. The pale yellow solid that precipitated was collected and recrystalized from 1,4-dioxane to give **18**. Yield: 82%; mp 320-322 °C. IR: 3083, 3020 (sp^2 CH); 2924 (sp^3 CH); 1678 (C=O), 1645 (C=N). ^1H NMR: 9.11 (s, 1H, pyrimidine-H); 7.07-7.83 (m, 10H: C^1H and ArH), 4.32 (d, *J* 8, 1H, C^4H); 2.88-2.92 (dd, J_1,J_2 = 8, 1H, C^3H); 2.44 (d, *J* 16, 1H, C^3H), 2.26 (s, 3H, CH_3); 1.83 (s, 3H, CH_3). Anal. Calcd. For $C_{28}H_{20}N_4OS_2$ (492.11): C: 68.27; H, 4.09; N, 11.37 %. Found: C: 68.05; H, 3.91; N, 10.98 %.

Biological activity. Determination of MIC of newly synthesized compounds

All synthesized compounds were evaluated as bactericidal agents and as antifungals following the dilution method.[36]

Preparation of cation adjusted MHB (20 mg Ca2+ and 10 mg Mg2+ per liter)

Prepare a 10 mg/ml (1000 mg/100 ml) Mg^{2+} stock solution by dissolving 8.36 g of $MgCl_2.6H_2O$ in 100 ml deionized water. Prepare a 10 mg/ml (1000 mg/100 ml) Ca^{2+} stock solution by dissolving 3.68 g of $CaCl_2.2H_2O$ in 100 ml deionized water. Filter-sterilize both stock solutions using 0.2-mm pore size cellulose-acetate filters. Prepare MHB according to the manufacturer's instructions, autoclave and cool the medium to 2 - 8 °C before the addition of the cation solutions. Add 100 ml of stock solution per 1 mg/L needed for 1 L of medium. For example, add 2 ml of Ca^{2+} stock solution if 20 mg needs to be added to 1 L MHB.

Preparation of inoculum

Using pure colonies from fresh agar plates to prepare bacterial suspension with a turbidity of 0.5 mcfarland ($1X10^8$ cfu/ml). Mix the bacterial suspension adjusted to $1X10^8$ cfu/ml and dilute it by a factor of 1:100 by adding 200 µl bacterial suspension to 19.8 ml sterile MHB in a sterile 25 ml Erlenmeyer flask to prepare a 20 ml inoculum of a concentration of $1X10^6$ cfu/ml. Each well containing the antibiotic solution (50 µl) and the

growth control (50 µl) well is intended to be supplied with with 50 µl of the bacterial suspension. This results in the final desired inoculum of $5X10^5$ cfu/ml.

Microtiter plate pipetting and testing

Each tested compound is first dissolved in DMSO to obtain a stock solution of 1024 µg/ml. Each row of 12 wells is desired to test only one compound (10 testing wells represent serial dilution of the tested compound and the last two wells represent negative control (sterility) and positive control (fertility)). The whole plate is desired to test a total of 8 compounds. Each testing well and positive control well is first supplied with 50 µl MHB. Each negative control well is supplied with 100 µl MHB. For each testing row, Take 50 µl from each tested compound stock solution in the first testing well to obtain a concentration of 512 µg/ml. From the first well of each row, transfer 50 µl to the second well to obtain a concentration of 256 µg/ml. Complete serial dilution of each compound by repeating the previous step till well 10 to obtain concentrations of each compound of 128, 64, 32, 16, 8, 4,2, and 1 µg/ml. Discard 50 µl from well 10 in each row. Finally, Add 50 µl of working bacterial suspension ($1X10^6$ cfu/ml) to each testing and growth control well to obtain final inoculum of $5X10^5$ cfu/ml in these well. Now, the final concentrations for each tested compound will be 256, 128, 64, 32, 16, 8, 4, 2, 1, and 0.5 µg/ml.

Acknowledgements

The authors extend their appreciation to Taif University, Saudi Arabia for supporting this work through Project Number (TU-DSPP-2024-150).

Supplementary Material

Copies of IR and ^{1}H NMR spectra of synthesized compounds are available in the supplementary material file associated with this manuscript.

References

1. Hu, S.; Chen, J.; Cao, J. X.; Zhang, S. S.; Gu, S. X.; Chen, F. E. *Bioorg. Chem.* **2023**, *136*, 106549. https://doi.org/10.1016/j.bioorg.2023.106549
2. Mahmoud, M. R.; Fatma, S. M.; Abu, E. A.; Mahmoud, F.I.; Mohamed, H. H.; Yasmeen, M. A. *Synth. Commun.* **2018**, *48*, 428-438. https://doi.org/10.1080/00397911.2017.1406520
3. Zhang, Y. L.; Deng, C. X.; Zhou, W. F.; et al. *J. Inorg. Biochem.* **2019**, *201*, 110820. https://doi.org/10.1016/j.jinorgbio.2019.110820
4. Suryawanshi, V.B.; Bondge, A. S.; Dawle, J. K.; Mathapati, S. R. *Polycycl. Arom. Comp.* 2021, 42, 4404. https://doi.org/10.1080/10406638.2021.1892777
5. Avci, F. G.; Atas, B.; Toplan, G.; Gurer, C.; Akbulut, B. S.; *Studies in Natural Products Chemistry* **2021**, *70*, 87. https://doi.org/10.1016/B978-0-12-819489-8.00016-8
6. Galán, A.; Moreno, L.; Párraga, J. *Bioorg. Med. Chem.* **2013**, *21*, 3221.

https://doi.org/10.1016/j.bmc.2013.03.042

7. Ding, C. F.; Qin, X. J.; Yu, H. F.; Liu, Y. P.; Wang, X. H.; Luo, X. D. *Tetrahedron Lett.* **2019**, *60*, 151135. https://doi.org/10.1016/j.tetlet.2019.151135
8. Du, K. C.; Liu, Y. X.; Zong, K. Q.; Wang, Y. M.; Li, J. H.; Meng, D. L. *Phytochemistry*, **2022**, *200*, 113240. https://doi.org/10.1016/j.phytochem.2022.113240
9. Zhiyuan, X.; Haixin, D.; Xin, F.; Yan, X.; Fadi, Z.; Huochun, Y.; Yuan, Z. Y.; Yongxia, G.; Jing, Z.; Gang, F. *Nat. Prod. Commun.* **2024**, *19*, 1. https://doi.org/10.1177/1934578X241226562
10. Alagumuthu, M.; Sathiyanarayanan, K. I.; Arumugam, S. *Int. J. Pharm. Sci.* **2015**, *7*, 200.
11. Akhtar, M.; Niu, J.; Zhu, Y. J. *Eur. J. Med. Chem.* **2023**, *256*, 115412. https://doi.org/10.1016/j.ejmech.2023.115412
12. Xu, M.; Wagerle, T.; Long, J. K.; Lahm, G. P.; Barry, J. D.; Smith, R. M. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 4026. https://doi.org/10.1016/j.bmcl.2014.06.004
13. Christopher, J. A.; Atkinson, F. L.; Bax, B. D.; Brown, M. J. B.; Champigny, A. C.; Chuang, T. T.; Jones, E. J.; Mosley, J. E.; Musgrave, J. R. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2230. https://doi.org/10.1016/j.bmcl.2009.02.098
14. Duncan, K. W.; Chesworth, R.; Borlack-Sjodin, P. A.; John, M.; Jin, H. L. *Bioorg. Med. Chem.* **2000**,*10*, 1477.
15. Kung, P. P.; Rui, E.; Bergqvist, S.; Bingham, P.; Braganza, J.; Collins, M.; Cui, M.; Diehl, W.; Dinh, D.; Fan, C.; Fantin, V. R.; Gukasyan, H. J.; Hu, W.; Huang, B.; Kephart, S.; Krivacic, C.; Kumpf, R. A.; Li, G.; Maegley, K. A.; McAlpine, I.; Nguyen, L.; Ninkovic, S.; Ornelas, M.; Ryskin, M.; Scales, S.; Sutton, S.; Tatlock, J.; Verhelle, D.; Wang, F.; Wells, P.; Wythes, M.; Yamazaki, S.; Yip, B.; Yu, X.; Zehnder, L.; Zhang, W. G.; Rollins, R. A.; Edwards, M. Kung, P. P.; Rui, E.; Bergqvist, S.; Bingham, P.; Braganza, J.; Collins, M.; Cui, M.; Diehl, W.; Dinh, D.; Fan, C.; Fantin, V. R.; Gukasyan, H. J.; Hu, W.; Huang, B.; Kephart, S.; Krivacic, C.; Kumpf, R. A.; Li, G.; Maegley, K. A.; McAlpine, I.; Nguyen, L.; Ninkovic, S.; Ornelas, M.; Ryskin, M.; Scales, S.; Sutton, S.; Tatlock, J.; Verhelle, D.; Wang, F.; Wells, P.; Wythes, M.; Yamazaki, S.; Yip, B.; Yu, X.; Zehnder, L.; Zhang, W. G.; Rollins, R. A.; Edwards, M. *J. Med. Chem.* **2016**, *59*, 8306. https://doi.org/10.1021/acs.jmedchem.6b00515
16. Novais, J. S.; Campos, V. R.; Silva, A. C. J. A.; De Souza, M. C. B. V.; Ferreira, V. F.; Keller, V. G. L.; Ferreira, M. O.; Dias, F. R. F.; Vitorino, M. I.; Sathler, P. C.; Santana, M. V.; Resende, J. A. L. C.; Castro, H. C.; Cunha, A. C. *RSC Adv.* **2017**, *7*, 18311. https://doi.org/10.1039/C7RA00825B
17. Kubo, A.; Nakahara, S.; Inaba, K.; Kitahara, Y. *Chem. Pharm. Bull.* **1986**, *34*, 4056.
18. Ryu, C. K.; Song, A. L.; Hong, J. A. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2065. https://doi.org/10.1016/j.bmcl.2013.01.130
19. Wenkert, E.; Dave, K. G.; Gnewuch, C.; Sprague, P. W. *J. Am. Chem. Soc.* **1968**, 5251. https://doi.org/10.1021/ja01021a033
20. Maeda, R.; Ohsugi, E. *Chem. Pharm. Bull.* **1968,** *16*, 897. https://doi.org/10.1248/cpb.16.897
21. Boyd, D. R.; Davies, R. J. H.; Hamilton, L.; McCullough, J. J. *J. Chem. Soc. Perkin Trans. 1,* **1992**, 31. https://doi.org/10.1039/p19920000031

22. Boyd, D. R.; Sharma, N. D.; Dorrity, M. R. J.; Hand, M. V; McMordie, R. A. S.; Malone, J. F. *J. Chem. Soc. Perkin Trans. I*, **1993**, 1065. https://doi.org/10.1039/p19930001065
23. Fukumi, H.; Kurihara, H.; Mishima, H. *Chem. Pharm. Bull.* **1978**, *26*, 2175. https://doi.org/10.1248/cpb.26.2175
24. Nagata, H.; Ochiai, K.; Aotani, Y.; Ando, K.; Yoshida, M.; Takahashi, I.; Tamaoki, T. *J. Antibiot. (Tokyo),* **1997**, *50*, 537. https://doi.org/10.7164/antibiotics.50.537
25. Oogose, K.; Hafuri, Y.; Takemori, E.; Nakata, E.; Inouye, Y. *J. Antibiot. (Tokyo)*, **1987**, *40*, 1778. doi: 10.7164/antibiotics.40.1778. https://doi.org/10.7164/antibiotics.40.1778
26. Bedard, J.; May, S.; Heureux, L. L.; Stamminger, T.; Copsey, A.; Drach, J.; Huffman, J.; Chan, L.; Jin, H.; Rando, R. F. *Antimicrob. Agents Chemother*. **2000**, *44*, 929. https://doi.org/10.1128/AAC.44.4.929-937.2000
27. Marae, I. S.; Bakhite, E. A.; Moustafa, O.; Abbady, M. S.; Mohamed, S. K.; Mague, J. T. *ACS Omega* **2021**, *6*, 8332. https://doi.org/10.1021/acsomega.1c00050
28. Marae, I. S.; Bakhite, E. A.; Moustafa, O.; Abbady, M. S.; Mohamed, S. K.; Mague, J. T. *ACS Omega* **2021**, *6*, 8706. https://doi.org/10.1021/acsomega.1c00601
29. Marae, I. S.; Sharmoukh, W.; Bakhite, E. A.; Moustafa, O. S.; Abbady, M. S.; Emam, H. E. *Cellulose* **2021**, *28*, 5937.
30. Al-Taifi, E. A.; Marae, I. S.; Bakhite, E. A.; Demirtas, G.; Mague, J. T.; Mohamed, S. K.; Ramli, Y. *Acta Cryst.* **2021**, *E77*, 121. https://doi.org/10.1107/S2056989021000372
31. Sayed, E. M.; Hassanien, R.; Mohamed, S. K..; Mague, J. T.; Akkurt, M.; Farhan, N.; Bakhite, E. A.; Al-Waleedy, S. A. H. *Acta Cryst.* **2021**, *E77*, 663. https://doi.org/10.1107/S2056989021005430
32. Akkurt, M.; Marae, I. S.; Mague, J. T.; Mohamed, S. K.; Bakhite, E. A.; Al-Waleedy, S. A. H. *Acta Cryst*. **2021**, *E77*, 527. *https://doi.org/10.1107/S2056989021003674*
33. Mague, J. T.; Al-Taifi, E. A.; Mohamed, S. K.; Akkurt, M.; Bakhite, E. A. *IUCr Data* **2017**, *2*, x170868. https://doi.org/10.1107/S2414314617008689
34. Mague, J. T.; Mohamed, S. K.; Akkurt, M.; Bakhite, E. A.; Albayati, M. R. *IUCr Data* **2017**, *2*, x170390. https://doi.org/10.1107/S241431461700390X
35. Marae, I. S.; Ibrahim, O. F.; Abdel-Hafez, Sh. H.; Mohamed, S. K.; Mague, J. T.; Bakhite, E. A. *J. Heterocycl. Chem*. **2022**, *59*, 1230. https://doi.org/10.1002/jhet.4462
36. Balouiri, M.; Sadiki, M.; Ibnsouda, S. K. *J. Pharm. Anal.* **2016**, *6*, 71-79. https://doi.org/10.1016/j.jpha.2015.11.005