Utility of Lauroyl Isothiocyanate as a Scaffold in the Synthesis of Some Novel Pyrimidine Derivatives and Their Antimicrobial Assessment

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The reaction of lauroyl isothiocyanate 1 with enaminonitrile derivative 2 furnished N-(6-cyano-3,4-diphenylthieno[2,3-c]pyrazidin-5-yl-carbamothioyl)dodecanamide 3, which was used as precursor for the synthesis of novel heterocyclic systems. Polyfunctional pyrimidine and fused pyrimidine derivatives were obtained by the cyclization of compound 3 under different basic conditions as well as its reactions with thiourea, o-aminothiophenol, hydrazine hydrate, phenyl hydrazine, ethyl phenyl acetate or ethyl benzoyl acetate. The structures of the new compounds were confirmed by microanalytical and spectral properties. The synthesised compounds were tested in-vitro for their antimicrobial activity and showed congruent results against most of the tested microorganisms compared to the standard drugs Gentamycin and Ketoconazol.

Key words lauroyl isothiocyanate; enaminonitrile; pyrimidine; fused pyrimidine; antimicrobial screening

Compounds containing enaminonitrile moiety are used as a good synthon for the construction of various heterocyclic systems, since they have both nucleophilic and electrophilic centers. These centers allow them to react with a variety of compounds via cyclization reactions to give fused heterocyclic compounds. In addition, isothiocyanates containing -N=C=S functional group that is of immense importance in organic synthetic strategies. Furthermore, the presence of carbonyl group in acyl isothiocyanates imparts unique reactivity to them; leading to the synthesis of biologically important heterocyclic skeletons such as thiazoles, benzimidazoles, triazoles, dithiolane, thiadiazoles, pyrimidines, fused pyrimidines, fused oxazolines, triazines, and oxazines.

Pyrimidine and fused pyrimidine derivatives are a class of important heterocycles that have attracted considerable attention due to their therapeutic and pharmacological properties such as anticancer, antiviral, antihypertensive, analgesic, antiinflammatory, antifungal and antibacterial.

In the light of previous observations and benefits, and in continuation of our previous work in developing synthetic strategies for synthesis of fused heterocyclic compounds, lauroyl isothiocyanate reacted with a starting material containing enaminonitrile moiety to give polynuclear heterocyclic compounds containing pyridazine, thiophene, pyrimidine, benzimidazole, 1,2,4-triazole, 1,3,5-triazine and 1,3-oxazine rings.

Results and Discussion

Reflexing a solution of lauroyl isothiocyanate 1 and 5-amino-3,4-diphenylthieno[2,3-c]pyrazidin-6-carbonitrile 2 in a dry acetonitrile furnished N-(6-cyano-3,4-diphenylthieno[2,3-c]pyrazidin-5-yl-carbamothioyl)dodecanamide 3 in a good yield; as illustrated in Chart 1. The spectral and microanalytical data of compound 3 agree with its proposed structure. The IR spectrum showed absorption bands at 3272 and 3175 cm⁻¹ due to the presence of two N–H groups, in addition to the presence of intense broad band at 1546 cm⁻¹ correlated with C–N–H vibration of combination bands due to N–H deformation and C–N stretching. Also, the IR spectrum shows stretching bands due to C–H com, C–H alk, C=N, C=C, C=O amide and C=S groups at 3063, 2920, 2850, 2214, 1692, 1628 and 1263 cm⁻¹, respectively. The MS spectrum of compound 3 revealed its molecular ion peak at (M⁺=569). The ¹H-NMR spectrum displayed signals of lauroyl and phenyl protons, as well as two singlet signals for two N–H protons, which were exchanged with D₂O (cf. Experimental).

Compound 3 is used as a building block to give new condensed pyrimidine derivatives. Treatment of compound 3 with an equivalent amount of freshly prepared sodium ethoxide in ethanol at room temperature afforded 1-(8-imino-3,4-diphenyl-6-thioxo-5,8-dihydropyrimido[4',5',4,5]-thieno[2,3-c]-pyridazin-7(6H)-yl)dodecan-1-one 4, while refluxing a solution of compound 3 in ethanol with sodium hydroxide (NaOH) produced the acid derivative 5. Interaction of compound 3 with piperidine in ethanol under reflux for 5 h afforded 1-(8-imino-3,4-diphenyl-6-(piperidin-1-yl)pyrimidido[4',5',4,5]-thieno[2,3-c]-pyridazin-7(8H)-yl)dodecan-1-one 6. Besides, refluxing a solution of compound 3 in ethanol with (3 mol) hydrochloric acid (HCl) yielded the fused 1,3-oxazine derivative 7 instead of the expected pyrimidine derivative 8, as illustrated in Chart 2. The spectral properties of the new products 4–7 agree with their proposed structures (cf. Experimental).

Treatment of compound 3 with thiourea in absolute ethanol in presence of catalytic amount of sodium hydroxide under reflux afforded 11-imino-3,4-diphenyl-9-undecylpyridazino[4",3";5'";4',5']thieno-[3',2'";4,5]-
pyrimido[1,2-α]-1,3,5-triazine-7(6H)-thione \(9\), while refluxing of compound \(3\) with \(o\)-amino-thiophenol in ethanol in presence of catalytic amount of piperidine yielded 3,4-diphenylpyrazino-[4,3-α:3,4-α]-thieno[3,2-b:4,5]-pyrimido[1,6-α]benzimidazole-6(5H)-thione \(10\). Moreover, condensation of \(3\) with hydrazine hydrate and/or phenyl hydrazine in ethanol under reflux gave 1,2,4-triazolo[4,3-α,1-β]-pyrimido[4,5,5'-4,5]thieno[2,3-c]pyridazine derivatives \(11a\) and \(11b\), respectively; as shown in (Chart 3).

Finally, reaction of compound \(3\) with ethyl phenyl acetate and/or ethyl benzoyl acetate afforded the polyfunctional pyrimidine derivatives 5-(5-substituted-6-hydroxy-2-thioxo-4-undecylpyrimidin-1-(2H)-yl)-3,4-diphenylthieno[2,3-c]-pyridazine-6-carbonitrile \(13a\) and \(13b\), respectively (Chart 4). The fused pyrimidine derivatives \(14a, b\) were not obtained as indicated from the microanalytical and spectral data. The structures of compounds \(9–13\) were established on the basis of their microanalytical and spectral data (cf. Experimental).

**Antimicrobial Evaluation**

Applying the agar plate diffusion technique,\(^{26,27}\) the antimicrobial activity of the new synthesised compounds were screened *in-vitro* against Gram-positive bacteria (G\(^+\)) namely *Staphylococcus aureus* and *Bacillus subtilis*, Gram-negative bacteria (G\(^-\)) namely *Escherichia coli* and *Proteus vulgaris* and fungi namely *Aspergillus fumigatus* and *Candida albii*.
cans) using the standard antibiotics Gentamycin (minimum inhibitory concentration (MIC) 4µg/mL) and Ketoconazole (MIC 100µg/mL). The compounds were dissolved in dimethyl sulfoxide (DMSO) at concentration of 10mg/mL. The inhibition zone was measured around each well after 24h at 37°C. Controls using DMSO were adequately done, the mean zone of inhibition in mm beyond well diameter 6mm produced on a range of the pathogenic microorganisms, results are depicted in Table 1.

The results revealed that only one compound 4 showed very high antimicrobial activity against the examined Gram-positive bacteria Staphylococcus aureus, and two compounds 9 and 10 showed very high antimicrobial activity against the examined Gram-positive bacteria Bacillus subtilis. On the other hand, six compounds 3, 7, 9, 10, 11b and 13b as well as three compounds 4, 11a and 11b showed high antimicrobial activity against the examined Gram-positive bacteria Staphylococcus aureus and Bacillus subtilis, respectively. Only compound 11b showed relatively high antimicrobial activity against the both examined Gram-positive bacteria Staphylococcus aureus and Bacillus subtilis.

In conclusion, results of antimicrobial activity revealed that the synthesised compounds showed moderate to high antimicrobial activity against the tested microorganisms comparable with the standard antibiotics Gentamycin and Ketoconazole, due to the formation of new polynuclear heterocyclic compounds containing pyrimidine moiety.

### Experimental

Melting points (mp) of the reaction products were determined in open capillary tubes on an electrothermal melting point apparatus and were uncorrected. The elemental analyses were performed on a Perkin-Elmer 2400 CHN elemental analyzer. The Fourier transform (FT) IR were recorded on a Perkin-Elmer Model 297 Infrared spectrometer using the KBr wafer technique. The 1H-NMR spectra were measured on a Varian Gemini 300MHz spectrometer, with chemical shift (δ) expressed in ppm downfield with tetramethylsilane (TMS) as internal standard, in DMSO-d6. Mass spectra were determined on a Shimadzu GC-MSQP 1000 EX instrument operating at 70eV. TLC was run using TLC aluminium sheets silica gel F254 (Merck). It was used in the monitoring of the progress of all reactions and in the checking of the homogeneity of the

### Table 1. Antimicrobial Activity of the Synthesised Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
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<tr>
<td></td>
<td>Staphylococcus aureus</td>
<td>Bacillus subtilis</td>
<td>Aspergillus fumigatus</td>
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<td></td>
<td>RCMB 010010</td>
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<td>RCMB 002008</td>
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<td>11b</td>
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<td>13a</td>
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<td>13b</td>
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<tr>
<td>Gentamycin</td>
<td>24</td>
<td>26</td>
<td>30</td>
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<tr>
<td>Ketoconazole</td>
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* Zone of inhibition: 0–12mm (low); 13–16mm (moderate); 17–20mm (high); >20mm (very high); NA=No activity; Well diameter of the hole=6.0mm (100µL was tested); RCMB: Regional Center for Mycology and Biotechnology.
synthesized compounds. The antimicrobial activity was studied at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Egypt.

5-Amino-3,4-diphenylthieno[2,3-c]pyridazine-6-carboxonitrile (2) Compound 2 was prepared according to literature.28,29

1-(8-Methyl-4,5-diphenylthieno[2,3-c]pyridazin-5-ylcarbamothioyl)dodecanamide (3) A solution of equimolar mixture of lauroyl isothiocyanate 1 (3 mmol, 0.72 mL) and 5-amino-4,5-diphenylthieno[2,3-c]pyridazin-6-carbonitrile 2 (3 mmol, 0.99 g) in a dry acetonitrile (30 mL) was added. The reaction mixture was heated under reflux for 1.5 h. A solid product that was obtained after cooling to room temperature was collected by filtration, dried and recrystallized from ethanol to give compound 3 as yellow crystals. Yield 81%; mp 222–224°C.1H-NMR (DMSO-d6): δ 0.84 (3H, CH3(CH2)8CH2CH2CO, t), 1.21–1.25 (16H, CH3(CH2)8CH2CH2CO, m), 1.40–1.45 (2H, CH2(CH2)3CH2CH2CO, m), 2.34 (2H, CH2(CH2)3CH2CH2CO, t), 7.23–7.48 (10H, two-Ph rings, m), 8.38 (1H, N HC=O), exchangeable with D2O, s), 10.26 (1H, O H, exchangeable with D2O, s). IR (KBr): ν: 0.85 (3H, CH3(CH2)8CH2CH2CO, t), 7.12–7.31 (10H, two-Ph rings, m), 7.99 (1H, C=NH, exchangeable with D2O, s). 1C-NMR (DMSO-d6): δ 137.75, 125.80, 124.80 (2C, phenyl-C), 120.58, 119.35, 116.30 (4C, phenyl-C), 126.00, 116.00 (2C, C=O, amide, NHC=O, exchangeable with D2O, s), 135.30, 129.00, 128.85 (4C, phenyl-C), 128.00 (2C, C=O, phenyl-C), 130.10, 129.00 (2C, phenyl-C, phenyl-C), 128.00 (4C, phenyl-C, phenyl-C), 127.95, 125.80, 45.40 (2C, piperidyl-C), 37.75, 34.40, 33.30 (3C, lauroyl-C), 33.35, 32.95, 32.80, 28.05, 25.15, 16.30. 1R (KBr) v: 3272, 3175 two (N–H), 3056 (C–H arom), 2926, 2853 (C–H alkyl), 1663 (C=Oamide), 1613 (C=Oamide), 1601 (C=Oamide), 1536 (C, phenyl-C), 1535 (C, phenyl-C), 1482 (C=Oamide), 1478 (C=Oamide), 1450 (C=Oamide), 1364 (C=Oamide).


1-(8-Methyl-3,4-diphenyl-6-(piperidin-1-yl)pyrimido[4′,5′:4,5]thieno[2,3-c]pyridazin-7(8H)-yl)-dodecan-1-one (6) An equivalent amount of compound 3 (3 mmol, 1.71 g) and piperidine (3 mmol, 0.30 mL) in absolute ethanol (30 mL) was refluxed for 5 h. The reaction mixture was cooled to room temperature. A precipitated solid was collected by filtration and recrystallized from ethanol to give compound 6 as brown crystals. Yield 61%; mp 140–142°C (decomp.).1H-NMR (DMSO-d6): δ 0.85 (3H, CH3(CH2)8CH2CH2CO, t), 1.19–1.28 (16H, CH3(CH2)8CH2CH2CO, m), 1.35–1.68 (8H, CH2(CH2)3CH2CH2CO), 2.94–2.96 (4H, 4 piperidyl-H, m), 7.12–7.31 (10H, two-Ph rings, m), 7.99 (1H, C=NH, exchangeable with D2O, s). 1C-NMR (DMSO-d6): δ 171.50 (C=O), 163.40 (C=NH), 163.00, 153.95, 139.55, 137.40, 132.80, 129.00, 128.85 (4C, phenyl-C), 128.00 (2C, phenyl-C, phenyl-C), 128.00 (4C, phenyl-C), 127.95, 125.80, 45.40 (2C, piperidyl-C), 37.75, 34.40, 33.30 (3C, lauroyl-C), 33.35, 32.95, 32.80, 28.50 (2C, piperidyl-C), 27.10 (1C, piperidyl-C), 26.70, 25.15, 16.30. IR (KBr) v: 3422 (N–H), 2926, 2853 (C–H alkyl), 1677 (C=Oamide), 1610 (C=N, C=O) cm−1. MS (70 eV) m/z (%): 620 (M+), 100, 423 (92.44), 396 (26.17), 294 (21.57), 262 (15.84), 224 (20.48), 85 (27.28). Anal. Calc. for C27H22N4O2S (620.83): C, 71.58; H, 7.14; N, 13.14. Found: C, 71.86; H, 7.18; N, 13.62%.

N-(3,4-Diphenyl-6-oxo-8H-pyrazidino[4′,3′:4,5]thieno[3,2-d]-1,3-oxazin-6-yl)dodecanamide (7) A solution of compound 3 (3 mmol, 1.71 g) in ethanol (30 mL), hydrochloric acid (3m, 5 mL) was added, and the reaction mixture was heated under reflux for 3 h. The solvent was evaporated under reduced pressure to a small volume. A solution of 0.1 N sodium carbonate was added until effervescence ceased. The solid precipitate was filtered off, dried and recrystallized from ethanol to give compound 7 as buff crystals. Yield 69%; mp 167–169°C.1H-NMR (DMSO-d6): δ 0.83 (3H, CH3(CH2)8CH2CH2CO, t), 1.22–1.26 (16H, CH3(CH2)8CH2CH2CO, m), 1.40–1.44 (2H, CH2(CH2)3CH2CH2CO, m), 2.34 (2H, CH2(CH2)3CH2CH2CO, t), 7.18–7.31 (10H, two-Ph rings, m), 8.04 (1H, NH, exchangeable with D2O, s). IR (KBr) v: 3180 (N–H), 3056 (C–H alkyl), 2924, 2854 (C–H alkyl), 1735 (C=Oamide), 1651 (C=Oamide), 1601 (C=N) cm−1. MS (70 eV) m/z (%): 554 (M+), 20.55, 539 (22.96), 483 (1569), 482 (27.62), 469 (12.22), 455 (9.59), 356 (11.14), 308 (100), 117 (0.99). Anal. Calc. for C37H31N4O2S (554.68): C, 69.29; H, 6.18; N, 10.10. Found: C, 69.47; H, 6.25; N, 10.18%.

11-Mino-3,4-diphenyl-9-undecyloxydipazidino[4′,3′:4,5]thieno[3′,2′:4,5]pyrimido[1,2-a][1,3,5]triazine-7(6H)-thione
A mixture of compound 3 (3 mmol, 1.71 g), thiourea (3 mmol, 0.23 g) and a catalytic amount of sodium hydroxide in absolute ethanol (30 mL) was refluxed for 3 h. A solid product was obtained after cooling, filtered off, washed with water and recrystallized from ethanol to give compound 9 as brown crystals. Yield 58%; mp 170–172°C (decomp.). 1H-NMR (DMSO-d6) δ: 0.85 (3H, CH3(CH2)8CH2CH2), 1.18–1.24 (16H, CH(CH2)8CH2CH2, m), 1.38–1.44 (4H, CH3(CH2)8CH2CH2, m), 7.18–7.45 (10H, two-Ph rings, m), 7.91 (1H, NH=S, exchangeable with D2O, s). IR (KBr) ν: 3347 (N–H), 124.40, 122.95 (2C), 115.35 (2C). IR (KBr) ν: 3312, 3175 two (N–H), 3058 (C–H arom), 2922, 2851 (C–Halkyl), 1616 (C=N), 1205 (C=S) cm⁻¹. MS (70 eV) m/z (%): 625 (M⁺, 37.28), 447 (7.68), 419 (25.62), 395 (14.97), 230 (10.48), 206 (100), 178 (10.71), 77 (19.57). Anal. Calcd for C32H35N7S: C, 73.92; H, 6.28; N, 15.64%.

General Procedure for the Preparation of Compounds 13a and 13b A solution of compound 3 (3 mmol, 1.71 g) and ethyl phenyl acetate and/or ethyl benzoyle acetate (3 mmol) in dry benzene was refluxed for 13 h in presence of triethyl amine (1.5 mL). The reaction mixture was cooled to room temperature. A solid product obtained was filtered off and recrystallized from benzene to give compounds 13a and 13b, respectively.

5-(6-Hydroxy-5-phenyl-2-thioxo-4-undecylpyrimidin-1-(2H)-yl)-3,4-diphenylthieno[2,3-c]pyridazine-6-carbonitrile (13a) Orange crystals, yield 63.5%; mp 179–181°C. 1H-NMR (DMSO-d6) δ: 0.84 (3H, CH3(CH2)8CH2CH2), 1.17–1.30 (18H, CH(CH2)8CH2CH2, m), 1.34 (2H, CH2(CH2)8CH2CH2, t), 7.12–7.48 (15H, three-Ph rings, m), 12.56 (1H, O–H, exchangeable with D2O, s). IR (KBr) ν: 3441 (O–H), 3056 (C–H=C=O), 2976, 2882 (C–Halkyl), 2180 (C=N), 1601 (C=N), 1236 (C=S) cm⁻¹. MS (70 eV) m/z (%): 669 (M⁺, 25.03), 652 (41.24), 551 (27.54), 394 (24.55), 347 (21.56), 328 (100), 322 (20.96), 275 (36.49), 118 (14.75). Anal. Calcd for C40H39N5O2S2: C, 71.71; H, 5.87; N, 10.46. Found: C, 71.99; H, 5.94; N, 10.57%.

5-(Benzyol-6-hydroxy-2-thioxo-4-undecylpyrimidin-1-(2H)-yl)-3,4-diphenylthieno[2,3-c]pyridazine-6-carbonitrile (13b) Orange crystals, yield 61%; mp 198–200°C. 1H-NMR (DMSO-d6) δ: 0.84 (3H, CH3(CH2)8CH2CH2), 1.08–1.31 (18H, CH(CH2)8CH2CH2, m), 1.34 (2H, CH2(CH2)8CH2CH2, t), 7.13–7.58 (15H, three-Ph rings, m), 12.85 (1H, O–H, exchangeable with D2O, s). IR (KBr) ν: 3347 (O–H), 3054 (C–H=C=O), 2979, 2887 (C–Halkyl), 2188 (C=N), 1667 (C=O), 1264 (C=S) cm⁻¹. MS (70 eV) m/z (%): 697 (M⁺, 100), 671 (25.30), 620 (29.10), 592 (28.74), 452 (29.50), 420 (70.22), 385 (36.09), 370 (34.41), 327 (27.47), 277 (36.49), 77 (55.33). Anal. Calcd for C40H39N5O2S2 (697.88): C, 70.56; H, 5.63; N, 10.04. Found: C, 70.89; H, 5.69; N, 10.16%.

Conflict of Interest The authors declare no conflict of interest.

References