Synthesis and characterization of new diiodocoumarin derivatives with promising antimicrobial activities

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Abstract

A series of 6,8-diiodocoumarin-3-N-carboxamides (4–11) were prepared. Treatment of ethyl 6,8-diiodocoumarin-3-carboxylate (1) with ethyl cyanoacetate/NH₄OAc gave ethyl 2-(3-carbamoyl-6,8-diiodocoumarin-4-yl)-2-cyanoacetate (12) and 2-amino-4-hydroxy-7,9-diiodocoumarin[3,4-c]pyridine-1-carbonitrile (13), and treatment with acetone in the presence of NH₄OAc or methyl-amine gave the ethyl 4-oxo-2,6-methano-2-methyl-3,4,5,6-tetrahydro-8,10-diiodo[2,1-g]-2H-1,3-oxazocine-5-carboxylate derivatives 14a,b. All compounds were evaluated for their antimicrobial activity and the compounds 12–14a,b exhibited a pronounced effect on all tested microorganisms.

Introduction

Coumarins and their derivatives are biologically and pharmaceutically interesting compounds known for their use as additives in food, perfumes, cosmetics, pharmaceuticals, platelet aggregation and agrochemicals [1,2]. Coumarins have also been reported to exhibit several biological activities, such as antimicrobial, anticancer, antifungal, anti-HIV and antioxidant properties [3-6], and they also served as versatile precursors for many organic transformations in the synthesis of a number of
drug-like molecules [7,8]. Moreover, coumarin-based dyes and pigments are organic fluorescent materials exhibiting unique photochemical and photophysical properties, which render them useful in a variety of applications such as dye lasers, anion sensors, organic light-emitting diodes and solar cells [9,10].

Iodo-organic derivatives have been widely used as diagnostic-imaging drugs (such as diatrizoate meglumine, diatrizoic acid, ioxidamide, iodoxanol, iohexol, iomeprol and iopamidol) and as amebicides [11,12]. The benzoxazocine derivatives have received considerable attention due to their pharmacological properties, such as their antidepressant, antithrombotic, antipsychotic (for the central nervous system, CNS) and anti-breast-cancer activities [13].

In view of the important biological properties of the diiodocoumarin derivatives and iodo-organic compounds as medical agents, we planned to synthesize some new diiodocoumarin derivatives bearing side chains with different structures, as such derivatives could possess interesting and useful biological properties.

Results and Discussion
Interaction of 3,5-diiodosalicylaldehyde with diethyl malonate according to the literature procedure [14,15] afforded ethyl 6,8-diiodocoumarin-3-carboxylate (1). Treatment of 1 with hot ethanolic KOH (10%) followed by acidification with HCl gave the corresponding 6,8-diiodocoumarin-3-carboxylic acid (2), which on treatment with SOCl₂ gave the 6,8-diiiodocoumarin-3-carbonyl chloride (3). Treatment of 1 with piperidine in boiling ethanol or with p-phenylenediamine in boiling AcOH afforded the 6,8-diiiodocoumarin-3-carboxamide derivatives 4 and 5, respectively. Interaction of 3 with glycine in dry benzene under reflux gave the new 6,8-diiiodocoumarin-3-N,N-dimethylcarboxamide (7) instead of 6,8-diiiodocoumarin-3-ylcarbonylglycine (6). The formation of compound 7 suggests that two glycine molecules react with 1 followed by the loss of ammonia and decarboxylation, furnishing the observed product (Scheme 1).

The structures of compounds 3–5 and 7 were confirmed by IR, ¹H NMR, ¹³C NMR and MS. The IR spectra for compound 3 showed 1774, 1718 cm⁻¹ (2 CO); for compound 4 1713, 1631 cm⁻¹ (2 CO); for compound 5 1718 cm⁻¹ (CO); and for compound 7 1722, 1635 cm⁻¹ (2 CO). ¹H NMR for compounds 3–5 and 7 showed δ at 7.64–8.70 ppm (s, 1H, H-4), and ¹³C NMR for compounds 3 and 5 showed δ at 143.2 and 147.2 ppm (C-4), respectively. The mass spectra of compounds 3 and 7 showed the corresponding molecular ion peaks at m/z 460 (M⁺, 2.6%) and m/z 469 (M⁺, 18.5%). The fragmentation pattern of compounds 3 and 7 are illustrated in Scheme 2.

**Scheme 1**: Synthesis of 6,8-diiodocoumarin derivatives 1–7.
Reactions of 3 with 4-aminophenylethanol or p-aminophenol, or with potentially bifunctional amino acids (anthranilic acid and p-aminophenylacetic acid), was successful, and the corresponding 6,8-diodocoumarin-3-carboxamide derivatives 8–11 were obtained (Scheme 3).

The structures of compounds 8–11 were established by IR, $^1$H NMR, $^{13}$C NMR and MS. The IR spectra of compound 8 showed 3287 cm$^{-1}$ (OH, NH) and 1719 cm$^{-1}$ (CO) and for compound 9 3217 cm$^{-1}$ (NH, OH) and 1720 cm$^{-1}$ (CO). $^1$H NMR for 8 showed $\delta$ at 3.01 (t, $J = 7.0$ Hz, 2H, Ar-CH$_2$),
3.56 (s, 1H, OH), 3.83 (t, \( J = 7.0 \) Hz, 2H, CH\(_2\)-OH), and 10.49 ppm (s, 1H, NH), and for compound 11 at 3.55 (s, 2H, CH\(_3\)), 8.71 (s, 1H, H-4), 10.10 (brs, 1H, NH), and 10.49 (s, 1H, OH). The \(^{13}\)C NMR for 11 showed \( \delta \) at 160 (CO \( \delta \) lactone), 163.4 (CONH), and 176.5 ppm (COOH). The mass spectra of compounds 8–11 provided additional evidence for the proposed structures.

As the C3–C4 olefinic bond in ethyl 6,8-diiodocoumarin-3-carboxylate (1) is activated by conjugation with electron-withdrawing carbonyl groups, the behavior of 1 towards activated methylene compounds under Michael reaction conditions was investigated. Thus, treatment of 1 with ethyl cyanooacetate/\( \text{NH}_4\text{OAc} \) in boiling ethanol afforded two reaction products. The insoluble reaction product was identified as ethyl 2-(3-carbamoyl-6,8-diiodocoumarin-4-yl)-2-cyanoacetate (12) and the soluble reaction product was identified as 2-amino-4-hydroxy-7,9-diiodocoumarino[3,4-\( c \)]pyridine-1-carbonitrile (13), which probably formed as a result of amide formation, dehydration and intramolecular cyclization (Scheme 4).

The structures of compounds 12 and 13 were established by IR, \(^1\)H NMR, \(^{13}\)C NMR and MS. The IR spectra of compound 12 showed 3309, 3277 cm\(^{-1}\) (NH\(_2\)), 2206 cm\(^{-1}\) (CN), and 1643 cm\(^{-1}\) (CO), while the \(^1\)H NMR for compound 13 showed \( \delta \) at 7.89 (brs, 2H, NH\(_2\)), and 9.06 (brs, 1H, OH). The spectral data of compound 13 confirmed its enol structure.

Reaction of 1 with acetone in the presence of \( \text{NH}_4\text{OAc} \) or methylamine at room temperature for 7 days gave 1,3-oxazocine-5-carboxylate derivatives (14a,b) [16-18] (Scheme 5). The formation of 14 indicates that the activated methylene compounds attack at the C3–C4 olefinic bond in 1 under Michael reaction conditions to yield a cyclic Michael adduct, which underwent hydrolysis by \( \text{NH}_3 \) or \( \text{MeNH}_2 \) and cyclization through the elimination of H\(_2\)O (Scheme 5).

The structure of compound 14a was established by \(^{13}\)C NMR, which showed \( \delta \) at 42.5 (CH\(_3\)c), 168.4 cm\(^{-1}\) (CONH), and 170 cm\(^{-1}\) (CO). The structures of all newly synthesized compounds were confirmed by IR, \(^1\)H NMR, \(^{13}\)C NMR and mass spectrometry.

The inhibitory effects of the synthetic compounds against these organisms are given in Table 1, Figure 1 and Figure 2. Among the series tested, compounds 12–14a,b exhibited excellent antibacterial activity, better than the standard ampicillin, against two species of Gram-positive bacteria, Staphylococcus aureus.
Scheme 5: Synthesis of 1,3-oxazocine derivatives 14a,b.

Table 1: Biological activity of the newly synthesized compounds.

| Compound no. | Gram-positive | Gram-negative | Fungi          |
|--------------|---------------|---------------|----------------|
|              | Staphylococcus aureus (NCTC-7447) | Bacillus cereus (ATCC-14579) | Escherichia coli (NCTC-10410) | Serratia marcescens (IMRU-70) | Aspergillus fumigatus (MTCC-3008) | Candida albicans (MTCC-227) |
| 1            | 10            | 11            | 15             | 10             | 9              | –              |
| 2            | 13            | 10            | –              | 13             | –              | 10             |
| 3            | 16            | 15            | 10             | 12             | 10             | 10             |
| 4            | 15            | 14            | 12             | 10             | –              | –              |
| 5            | 10            | 12            | –              | 15             | 10             | –              |
| 7            | 10            | 10            | –              | 15             | 11             | –              |
| 8            | 20            | 18            | 14             | 10             | 16             | 15             |
| 9            | 22            | 22            | 22             | 17             | 14             | 13             |
| 10           | 22            | 15            | 22             | 15             | 17             | 11             |
| 11           | 20            | 22            | 20             | –              | 15             | 12             |
| 12           | 26            | 27            | 28             | 26             | 16             | 18             |
| 13           | 27            | 28            | 28             | 26             | 17             | 17             |
| 14a          | 26            | 28            | 28             | 26             | 15             | 14             |
| 14b          | 25            | 26            | 25             | 27             | 18             | 15             |
| Ampicillin   | 22            | 22            | 22             | 22             | –              | –              |
| Calforan     | –             | –             | –              | –              | 20             | 20             |

\( a = 1 \text{ mg mL}^{-1} \) in DMF.

(NCTC-7447), *Bacillus cereus* (ATCC-14579) and two Gram-negative bacteria, *Escherichia coli* (NCTC-10410) and *Serratia marcescens* (IMRU-70), while the same compounds showed moderate antifungal activity against the tested organisms. Compounds 9–11 exhibited comparable activity to ampicillin against the tested bacteria and moderate to weak antifungal activity against the tested organisms. Furthermore, compounds 1–8 showed moderate to weak activities against all the tested bacteria and fungi, compared with the standards ampicillin and calforan. In addition, compounds 2 and 5 in the series were found to be inactive against *Escherichia coli* (NCTC-10410), while compound 11 was inactive against *Serratia marcescens*.
Chemical shifts (δ) are related to that of the solvent. Mass spectra were measured on a Shimadzu GMMS-QP-1000 EX mass spectrometer at 70 eV. The elemental analyses were performed at the Microanalytical Center, Cairo University, Cairo (Egypt).

**Ethyl 6,8-diiodocoumarin-3-carboxylate (1).** Ethyl 6,8-diiodocoumarin-3-carboxylate (1) was prepared by the interaction of 3,5-diiodosalicylaldehyde with diethyl malonate according to the literature procedures [19-21].

**6,8-Diiodocoumarin-3-carboxylic acid (2).** A solution of compound 1 (0.47 g, 10 mmol) in absolute ethanol (20 mL) was mixed with ethanolic solution of KOH (10%), which was then refluxed for 10 min. The reaction mixture was poured onto ice, acidified with HCl and recrystallized from ethanol [22].

**6,8-Diiodocoumarin-3-carbonyl chloride (3).** Compound 2 (0.44 g, 10 mmol) was dissolved in dry benzene (40 mL), 2 mL of thionyl chloride was added and the solution was refluxed for 1 h. A few drops of formic acid were added to eliminate the unreacted thionyl chloride, and the solvent was removed under reduced pressure. The solid obtained was recrystallized from benzene. Yellow crystals: Yield 92%; mp 180 °C; Anal. calcd for C_{10}H_{3}ClI_{2}O_{3}: C, 26.10; H, 0.65; found: C, 26.11; H, 0.67; IR (KBr, cm\(^{-1}\)): 3055 (C–H aromatic), 1774, 1718 (2 CO); \(^1\)H NMR (300 MHz, CDCl\(_3\), δ/ppm) 8.01 (d, J = 1.8 Hz, 1H, Ar-H-7), 8.44 (d, J = 1.8 Hz, 1H, Ar-H-5), 8.58 (s, 1H, H-4); \(^1\)C NMR (75 MHz, CDCl\(_3\), δ/ppm) 86.0 (C-8), 89.1 (C-6), 118.6 (C-3), 120.3 (C-4a), 138.2 (C-5), 147.2 (C-4), 149.4 (C-7), 153.6 (C-8a), 155.0 (CO δ lactone), 162.9 (CO); MS m/z (% relative intensity): 460 (M\(^+\), 2.6), 459 (M – 1, 30.4), 425 (83.4), 341 (19.3), 214 (10.9), 87 (100).

**6,8-Diiodo-3-(piperidine-1-carbonyl)coumarin (4).** A solution of compound 1 (0.47 g, 10 mmol) in absolute ethanol (30 mL) was refluxed with piperidine (0.9 g, 10 mmol) for 1 h. After cooling, the solid formed was filtered off, washed with ethanol and dried under vacuum. The solid obtained was recrystallized from benzene. Colorless crystals: Yield 80%; mp 230 °C; Anal. calcd for C_{15}H_{13}I_{2}NO_{3}: C, 35.37; H, 2.55; N, 2.75; found: C, 35.36; H, 2.53; N, 2.76; IR (KBr, cm\(^{-1}\)): 3040 (C–H aromatic), 2935, 2854 (C–H aliphatic), 1713, 1631 (CO); \(^1\)H NMR (300 MHz, CDCl\(_3\), δ/ppm) 1.59, 1.67, 3.29, 3.69 (m, 10H, (CH\(_2\)\(_5\))), 7.64 (s, 1H, H-4), 7.78 (d, J = 2.1 Hz, 1H, Ar-H-7), 8.28 (d, J = 2.1 Hz, 1H, Ar-H-5); \(^1\)C NMR (75 MHz, CDCl\(_3\), δ/ppm) 29.35, 2854 (C–H aliphatic), 1713, 1631 (CO); IR (KBr, cm\(^{-1}\)): 3040 (C–H aromatic), 2935, 2854 (C–H aliphatic), 1713, 1631 (CO); \(^1\)H NMR (300 MHz, CDCl\(_3\), δ/ppm) 1.59, 1.67, 3.29, 3.69 (m, 10H, (CH\(_2\)\(_5\))), 7.64 (s, 1H, H-4), 7.78 (d, J = 2.1 Hz, 1H, Ar-H-7), 8.28 (d, J = 2.1 Hz, 1H, Ar-H-5); \(^1\)C NMR (75 MHz, CDCl\(_3\), δ/ppm) 24.30, 25.40, 48.03 (CH\(_2\) piperidine), 86.0 (C-8), 89.1 (C-6), 118.6 (C-3), 120.3 (C-4a), 138.2 (C-5), 147.2 (C-4), 149.4 (C-7), 153.6 (C-8a), 155.0 (CO δ lactone), 162.9 (CO-amide); MS m/z (% relative intensity): 509 (M\(^+\), 0.3), 424 (3.4), 341 (2.7), 214 (1.5), 84 (100).
N-(4-Acetamidophenyl)-6,8-diiodocoumarin-3-carboxamide (5). A solution of compound 1 (0.47 g, 10 mmol) in glacial acetic acid (30 mL) was refluxed with p-phenylenediamine (1.10 g, 10 mmol) for 2 h. After cooling, the solid formed was filtered off, washed with ethanol, dried under vacuum and recrystallized from benzene. Colorless crystals: Yield 87%; mp 291 °C; Anal. calcd for C₂₉H₂₃NO₅: C, 38.51; H, 2.32; N, 2.50; found: C, 38.52; H, 2.34; N, 2.51; IR (KBr, cm⁻¹): 3285 (OH, NH), 3049 (Ar-H), 2958, 2928 (aliphatic-H), 1719 (CO); ¹H NMR (300 MHz, DMSO-d₆, δ/ppm) 3.01 (t, J = 7.0 Hz, 2H, Ar-CH₂), 3.56 (s, 1H, OH), 3.83 (t, J = 7.0 Hz, 2H, CH₂-OH), 7.29, 7.63 (2d, J = 8.2 Hz, 4H, AB-q, Ar-H), 8.37 (d, J = 2.0 Hz, 1H, H-7), 8.46 (d, J = 2.0 Hz, 1H, H-5), 8.71 (s, 1H, H-4), 10.49 (s, 1H, NH); ¹³C NMR (75 MHz, DMSO-d₆, δ/ppm) 38.4 (Ar-CH₂), 62.2 (CH₂-OH), 111.2, 114.0 (C-6,8), 114.5, 119.8 (C-3',5',6'), 129.3, 132.1 (C-5,7), 135.4, 135.9, 136.0 (C-3',1',4'), 148.3 (C-4), 156.4, 159.9 (C=O, Ar-C=O, Ar-C=O), 163.9 (CO δ lactone), 163.9 (CO-amide); MS m/z (% relative intensity): 561 (M⁺, 0), 543 (M – H₂O, 3), 530 (67), 425 (M – NH-C₆H₄-CH₂CH₂OH, 100), 341 (6), 107 (36), 128 (23), 127 (14), 87 (36).

N-(4-Hydroxyphenyl)-6,8-diiodocoumarin-3-carboxamide (9). Yellow crystals: Yield 85%; mp 303 °C; Anal. calcd for C₁₄H₁₂O₅N: C, 46.1; H, 2.93; N, 2.76; found: C, 46.05; H, 2.94; N, 2.72; IR (KBr, cm⁻¹): 3217 (NH, OH), 1720 (CO); ¹H NMR (300 MHz, DMSO-d₆, δ/ppm) 7.25–7.85 (m, 4H, Ar-H), 8.35 (d, J = 1.8 Hz, 1H, Ar-H), 8.43 (d, J = 1.8 Hz, 1H, Ar-H), 8.70 (s, 1H, H-4), 10.12 (brs, 1H, OH), 11.9 (brs, 1H, NH); ¹³C NMR (75 MHz, DMSO-d₆, δ/ppm) 89.0 (C-6), 90.1 (C-8), 121.5, 128.0 (C-2', 3', 5', 6'), 114.3, 133.5, 135.6 (C-3, 1', 4'), 125.3 (C-4a), 136.2 (C-5), 143.2 (C-4), 146.4 (C-7), 148.6 (C-8a), 160.4 (CO δ lactone), 170.0 (CO(CH)), MS m/z (% relative intensity): 574 (M⁺, 3), 532 (M – CH₂–C=O, 38.7), 424 (18.2), 341 (33.8), 298 (65.5), 171 (9.3), 107 (100).

6,8-Diiodocoumarin-3-N,N-dimethylcarboxamide (7). A solution of compound 3 (0.46 g, 1 mmol) in dry benzene (50 mL) was refluxed with glycine (0.75 g, 10 mmol) for 2 h. After cooling, the solid formed was filtered off, washed with ethanol, dried under vacuum, and recrystallized from dioxane. Colorless crystals: Yield 83%; mp 302 °C; Anal. calcd for C₁₅H₁₄O₅N: C, 50.2; H, 3.60; N, 2.56; found: C, 50.60; H, 3.60; N, 2.56; IR (KBr, cm⁻¹): 3286 (OH, NH), 3047 (Ar-H), 2958 (aliphatic-H), 1719 (CO); ¹H NMR (300 MHz, DMSO-d₆, δ/ppm) 3.01 (t, J = 7.0 Hz, 2H, Ar-CH₂), 3.56 (s, 1H, OH), 3.83 (t, J = 7.0 Hz, 2H, CH₂-OH), 7.92, 7.63 (2d, J = 8.2 Hz, 4H, AB-q, Ar-H), 8.37 (d, J = 2.0 Hz, 1H, H-7), 8.46 (d, J = 2.0 Hz, 1H, H-5), 8.71 (s, 1H, H-4), 10.49 (s, 1H, NH); ¹³C NMR (75 MHz, DMSO-d₆, δ/ppm) 38.4 (Ar-CH₂), 62.2 (CH₂-OH), 111.2, 114.0 (C-6,8), 114.5, 119.8 (C-3',5',6'), 129.3, 132.1 (C-5,7), 135.4, 135.9, 136.0 (C-3',1',4'), 148.3 (C-4), 156.4, 159.9 (C=O, Ar-C=O), 163.9 (CO δ lactone), 163.9 (CO-amide); MS m/z (% relative intensity): 561 (M⁺, 0), 543 (M – H₂O, 3), 530 (67), 425 (M – NH-C₆H₄-CH₂CH₂OH, 100), 341 (6), 107 (36), 128 (23), 127 (14), 87 (36).

General procedure for the synthesis of 6,8-diiodocoumarin-3-carboxamide derivatives 8–11
To a well-stirred solution of 3 (0.46 g, 1 mmol) in dry dichloromethane (DCM) containing a few drops of triethylamine (TEA) an equivalent amount of an ambient nucleophile [4-aminofernilenethanol, p-aminophenol, anharinic acid and p-aminophenylacetic acid (1.2 mmol)] was added. The reaction mixture was stirred at room temperature under dry conditions for 3 h. DCM was removed under reduced pressure until dryness, the obtained solid was then washed with 10% HCl and the remaining solid recrystallized from dioxane.

N-(4-(2-Hydroxyethyl)phenyl)-6,8-diiodocoumarin-3-carboxamide (8). Yellow crystals: Yield 93%; mp 285 °C; Anal. calcd for C₁₈H₁₁₂O₅N: C, 37.57; H, 1.91; N, 2.44; found: C, 37.59; H, 1.92; N, 2.46; IR (KBr, cm⁻¹): 3286 (OH, NH), 3047 (Ar-H), 2916 (aliphatic-H), 1720 (CO); ¹H NMR (300 MHz, DMSO-d₆, δ/ppm) 3.55 (s, 2H, CH₂), 7.27, 7.64 (2d, J = 8.4 Hz, 4H, AB-q,
both cases a colorless solid formed after the solvent had evapo-
rate under reduced pressure, and the products were identified
as compounds 14a and 14b. The crude products were crystal-
lized from benzene.

Ethyl 4-oxo-2,6-methano-2-methyl-3,4,5,6-tetrahydro-8,10-
diidoibenzo[2,1-g]-2H-1,3-oxazocine-5-carboxylate (14a).
Colorless: Yield 72%; mp 222 °C; Anal. calcd. for C_{16}H_{12}I_{2}NO_{4}; C, 33.57; H, 1.89; N, 2.61; found: C, 33.81; H, 2.01; N, 2.67; IR (KBr, cm^-1): 3217 (NH), 2977 (aliphatic-H), 2206 (CN), 1643 (CO); ^1H NMR (300 MHz, DMSO-d_6, ppm) 1.23 (t, J = 7.2 Hz, 3H, CH_3(c)), 1.78 (s, 3H, CH_3(f)), 2.83 (3H, NCH_3), 3.28–3.42 (m, 2H, CH_2(b)), 3.4–3.57 (m, 2H, H(a) + H(b)), 4.18 (q, J = 7.2 Hz, 2H, CH_2(d)), 7.66 (d, 1H, Ar-H-9), 7.95 (d, J = 1.8 Hz, 1H, Ar-H-7); MS m/z (% relative intensity): 541 (M^+, 3.4), 196 (59.2), 150 (27.6), 56 (100).

Antimicrobial assays

The newly synthesized compounds were screened for their anti-
microbial activities in vitro against two species of Gram-positive
bacteria, namely Staphylococcus aureus (NCTC-7447),
Bacillus cereus (ATCC-14579), and two Gram-negative bacteria,
namely Escherichia coli (NCTC-10410), Serratia marcescens (IMRU-70); and against two species of fungi, namely Aspergillus fumigatus (MTCC-3008) and Candida albicans (MTCC-227). The tested microorganisms were obtained from the Regional Center for Mycology & Biotechnology (RCMP), Al-Azhar University.

The activities of these compounds were tested by using the disc-
diffusion method [23] for bacteria and the paper-disk-diffusion
method [24] for fungi. The area of zone inhibition was measured with ampicillin (30 µg mL^-1) as the standard anti-biotic reference for antibacterial activity, and calforan (30 µg mL^-1) was used as a reference antifungal activity. The

General procedure for the synthesis of ethyl
cyanooxycarbonyl derivatives 12

Ethyl 2-(3-carbamoyl-6,8-diiodocoumarin-4-yl)-2-cyano-
acetate (12). Pale yellow crystal: Yield 82%; mp 198 °C; Anal.
calcd. for C_{16}H_{12}I_{2}NO_{4}; C, 33.57; H, 1.89; N, 2.61; found: C, 33.81; H, 2.01; N, 2.67; IR (KBr, cm^-1): 3217 (NH), 2977 (aliphatic-H), 2206 (CN), 1643 (CO); ^1H NMR (300 MHz, DMSO-d_6, ppm) 1.23 (t, J = 7.2 Hz, 3H, CH_3(c)), 1.78 (s, 3H, CH_3(f)), 2.83 (3H, NCH_3), 3.28–3.42 (m, 2H, CH_2(b)), 3.4–3.57 (m, 2H, H(a) + H(b)), 4.18 (q, J = 7.2 Hz, 2H, CH_2(d)), 7.66 (d, 1H, Ar-H-9), 7.95 (d, J = 1.8 Hz, 1H, Ar-H-7); MS m/z (% relative intensity): 541 (M^+, 3.4), 196 (59.2), 150 (27.6), 56 (100).

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The newly synthesized compounds were screened for their anti-
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General procedure for the synthesis of ethyl
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Ethyl 2-(3-carbamoyl-6,8-diiodocoumarin-4-yl)-2-cyano-
acetate (12). Pale yellow crystal: Yield 82%; mp 310 °C; Anal.
calcd. for C_{16}H_{12}I_{2}NO_{4}; C, 32.62; H, 1.81; N, 5.07; found: C, 32.64; H, 1.79; N, 5.05; IR (KBr, cm^-1): 3309, 3277 (NH_2), 2206 (CN), 1643 (CO); ^1H NMR (300 MHz, DMSO-d_6, ppm) 1.50 (t, J = 7.2 Hz, 3H, CH_3), 4.44 (q, J = 7.2 Hz, 2H, CH_2), 5.05 (s, 1H, CH), 8.00 (d, J = 1.8 Hz, 1H, Ar-H-7), 8.40 (d, J = 1.8 Hz, 1H, Ar-H-5), 8.70 (brs, 2H, NH_2, exchangeable with D_2O); MS m/z (% relative intensity): 552 (M^+, 2), 388 (8.0), 313 (30.0), 264 (4.0), 236 (35.0).

2-Amino-4-hydroxy-7,9-diiodocoumarin[3,4-c]pyridine-1-
carbonitrile (13). Pale yellow crystals: Yield 84%; mp 340 °C; Anal.
calcd. for C_{16}H_{12}I_{2}NO_{4}; C, 30.90; H, 0.99; N, 8.32; found C, 30.92; H, 1.00; N, 8.34; IR (KBr, cm^-1): 3374 (OH), 3277, 3228 (NH_2), 2207 (CN), 1707, 1662 (CO); ^1H NMR (300 MHz, DMSO-d_6, ppm) 9.06 (brs, 1H, OH, exchangeable with D_2O), 8.20 (s, 1H, H-8), 7.97 (s, 1H, H-10), 7.89 (brs, 2H, NH_2, exchangeable with D_2O); MS m/z (% relative intensity): 505 (M^+, 100), 477 (M – CO, 18.9), 397 (20.8), 341 (18.9), 171 (25.2), 106 (32.6) and 87 (35.5).

General procedure for the synthesis of 1,3-
oxazocine-5-carboxylate derivatives 14a,b

A mixture of compound 1 (2.35 g, 5 mmol), acetone (30 mL) and (a) ammonium acetate (0.4 g, 5 mmol) or (b) methyamine (0.16 g, 5 mmol) was stirred at room temperature for 7 days. In both cases a colorless solid formed after the solvent had evapo-
rate under reduced pressure, and the products were identified
as compounds 14a and 14b. The crude products were crystal-
lized from benzene.

Ethyl 4-oxo-2,6-methano-2-methyl-3,4,5,6-tetrahydro-8,10-
diidoibenzo[2,1-g]-2H-1,3-oxazocine-5-carboxylate (14a).
Colorless: Yield 72%; mp 222 °C; Anal. calcd. for C_{16}H_{12}I_{2}NO_{4}; C, 33.57; H, 1.89; N, 2.61; found: C, 33.81; H, 2.01; N, 2.67; IR (KBr, cm^-1): 3217 (NH), 2977 (aliphatic-H), 2206 (CN), 1643 (CO); ^1H NMR (300 MHz, DMSO-d_6, ppm) 1.23 (t, J = 7.2 Hz, 3H, CH_3(c)), 1.78 (s, 3H, CH_3(f)), 2.83 (3H, NCH_3), 3.28–3.42 (m, 2H, CH_2(b)), 3.4–3.57 (m, 2H, H(a) + H(b)), 4.18 (q, J = 7.2 Hz, 2H, CH_2(d)), 7.66 (d, 1H, Ar-H-9), 7.95 (d, J = 1.8 Hz, 1H, Ar-H-7); MS m/z (% relative intensity): 541 (M^+, 3.4), 196 (59.2), 150 (27.6), 56 (100).
tested compounds were dissolved in \textit{N,N}-dimethylformamide (DMF) to give a solution of 1 mg mL$^{-1}$. The inhibition zones (diameter of the hole) were measured in millimeters (6 mm) at the end of an incubation period of 48 h at 28 °C; \textit{N,N}-dimethylformamide showed no inhibition zone.

**Conclusion**

It was interesting to note that four of the new compounds (12, 13 and 14a,b) were found to have an antimicrobial activity greater than that of the standard antibiotic claforan, while compounds 1-11 were either inactive or only weakly active against the tested microorganisms. The presence of fused diiodocoumarin[3,4-c]pyridine and diiodobenzo[2,1-g]pyridine isms. The presence of fused diiodocoumarinoxazocine nucleus increased the antimicrobial activity, whereas the presence of diiodocoumarin-3-carboxamides decreased the antimicrobial activity.

**References**

1. O’Kennedy, R.; Thomes, R. D.; Coumarins. Biology, Applications and Mode of Action; Wiley & Sons: Chichester, UK, 1997.
2. Zahradnik, M. The Production and Application of Fluorescent Brightening Agents; Wiley & Sons: Chichester, 1992.
3. Bailly, C.; Bal, C.; Barbier, P.; Combes, S.; Finet, J.-P.; Hildebrand, M.-P.; Peyrol, V.; Wattez, N. J. Med. Chem. 2003, 46, 5437–5444. doi:10.1021/jm0309003d
4. Yeh, J.-Y.; Coumar, M. S.; Horng, J.-T.; Shiao, H.-Y.; Kuo, F.-M.; Lee, H.-L.; Chen, I.-C.; Chang, C.-W.; Tang, W.-F.; Tseng, S.-N. J. Med. Chem. 2010, 53, 1519–1533. doi:10.1021/jm901570x
5. Pierson, J.-T.; Dumètre, A.; Hutter, S.; Delmas, F.; Laget, M.; Finet, J.-P.; Azas, N.; Combes, S. Eur. J. Med. Chem. 2010, 45, 864–869. doi:10.1016/j.ejmech.2009.10.022
6. Basile, A.; Sorbo, S.; Spadaro, V.; Bruno, M.; Maggio, A.; Faraone, N.; Rosselli, S. Molecules 2009, 14, 939–952. doi:10.3390/molecules14030939
7. Zhao, P.-L.; Wang, L.; Zhu, X.-L.; Huang, X.; Zhan, C.-G.; Wu, J.-W.; Yang, G.-F. J. Am. Chem. Soc. 2010, 132, 185–194. doi:10.1021/ja905756c
8. Teichert, J. F.; Feringa, B. L. Chem. Commun. 2011, 47, 2679–2681. doi:10.1039/c0cc05160h
9. Key, J. A.; Kho, S.; Timerghazin, Q. K.; Brown, A.; Cairo, C. W. Dyes Pigm. 2009, 82, 196–203. doi:10.1016/j.dyepig.2009.01.001
10. Zhou, S.; Jia, J.; Gao, J.; Han, L.; Li, Y.; Sheng, W. Dyes Pigm. 2010, 86, 123–128. doi:10.1016/j.dyepig.2009.12.005
11. Craven, P. A.; Pfanspiel, J.; DeRubertis, F. R. J. Clin. Invest. 1986, 77, 850–859. doi:10.1172/JCI112382
12. Ralthbone, D. L.; Su, D.; Wang, Y.; Billington, D. C. Tetrahedron Lett. 2000, 41, 123–126. doi:10.1016/S0040-4039(99)02027-4
13. Mishra, J. K.; Samanta, K.; Jain, M.; Dikshit, M.; Panda, G. Med. Chem. Lett. 2010, 20, 244–247. doi:10.1016/j.mcl.2009.10.126
14. Burton, H. J. Chem. Soc. 1945, 280, 280–283. doi:10.1039/JR945000280
15. Bonsignore, L.; Cottiglia, F.; Maccioni, A. M.; Sacci, D.; Lavagna, S. M. J. Heterocycl. Chem. 1995, 32, 573–577. doi:10.1002/jhet.5570320234
16. Koelsch, C. F. J. Am. Chem. Soc. 1945, 67, 569–574. doi:10.1021/ja12202a023
17. Koelsch, C. F.; Freekers, M. C. J. Org. Chem. 1953, 18, 1538–1545. doi:10.1021/jo50017a013
18. Bedair, A. H.; Aly, F. M.; El-Agrody, A. M.; El-Assy, R. K. M. Acta Pharm. 1986, 36, 363–369.
19. Kadnikov, D. V.; Larock, R. C. J. Organomet. Chem. 2003, 687, 425–435. doi:10.1016/S0022-328X(03)00786-1
20. Pottar, M. K.; Mohile, S. S.; Salunkhe, M. M. Tetrahedron Lett. 2001, 42, 9285–9287. doi:10.1016/S0040-4039(01)02041-X
21. Yavari, I.; Adib, M.; Hojabri, L. Tetrahedron 2002, 58, 6895–6899. doi:10.1016/S0040-4020(02)00758-5
22. Creaven, B. S.; Egan, D. A.; Kavanagh, K.; McCann, M.; Noble, A.; Thati, B.; Walsh, M. Inorg. Chem. Acta 2006, 359, 3976–3984. doi:10.1016/j.ica.2006.04.006
23. European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). Clin. Microbiol. Infect. 2000, 6, 509–515.
24. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed; Approved Standard M7-A5.Wayne, PA: NCCLS, 2000.