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Synthesis and Reactions of Some New Diiodocoumarin Derivatives Bearing Side Chains and Some of Their Biological Activities

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Abstract The synthesis of 6,8-diiodocoumarin derivatives (2-6) by condensation of 3,5-diiodosalicylaldehyde (1) with active methylene compounds is described. Reaction of 6 with malononitrile afforded two products pyridine and ethylidine malononitrile derivatives (7, 8), while treatment of 6 with *p*-methoxybenzylidenemalononitrile in absolute ethanol/piperdine provided pyrane and bis coumarin derivatives (9,10). Reaction of 6 with DMF/$POCl_3$ afforded three products coumarin derivatives (11,12,13), while bromination of 6 with Br_2/AcOH gave 3-bromo-6,8-diiodocoumarin (14). Treatment 6 with aromatic aldehydes gave the corresponding 3-(3-arylacryloyl)-6,8-diiodocoumarin derivatives (16a,b) while reaction of 6 with aniline or phenylhydrazine gave coumarin derivatives 17 and 18. Reaction of 18 with DMF/$POCl_3$ gave pyrazole-4-aldehyde derivative (19). Condensation of 19 with active methylene compounds afforded pyrazole and pyrimidine derivatives (20a-c) and 21). Reaction of 8 with sulphur via the Gewald reaction afforded thiophene derivative (22), while the reaction of 8 with *p*-methoxybenzylidenemalononitrile gave biphenyl derivative (23). Treatment of 8 with chloroacetyl chloride afforded the furan derivative (24). The structures of the newly synthesized compounds were confirmed by IR, ^{1}H NMR and ^{13}C NMR and mass spectral data. The newly synthesized compounds were also screened for their antimicrobial activity.

Keywords 3,5-Diiodosalicylaldehyde, Active Methylene Compounds, Aldehydes, Barbituric Acid, Vilsmeier Reaction, *p*-methoxybenzylidenemalononitrile, Antimicrobial Activity

1. Introduction

Coumarins and its derivatives have been used as additives in food, perfumes, cosmetics, pharmaceuticals, platelet aggregation, agrochemicals[1,2]. Coumarins have also been reported to exhibit several biological activities such as antimicrobial and anticancer, antifungal, anti-HIV, and anti-clotting[3-6], but also served as versatile precursors of 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].

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Iodo-organic derivatives have been widely used as diagnostic imaging drugs (such as diatrizoate meglumine, diatrizoic acid, iodipamide, iodixanol, iohexol, iomeprol and iopamidol) and other as amebicides[11,12]. In view of the important biological properties of the coumarin derivatives and iodo-organic compounds as medicinal agents, it was planned to the synthesis of some newly diiodocoumarin derivatives bearing side chains with different structural features. Such derivatives could possess interesting and useful biological properties.

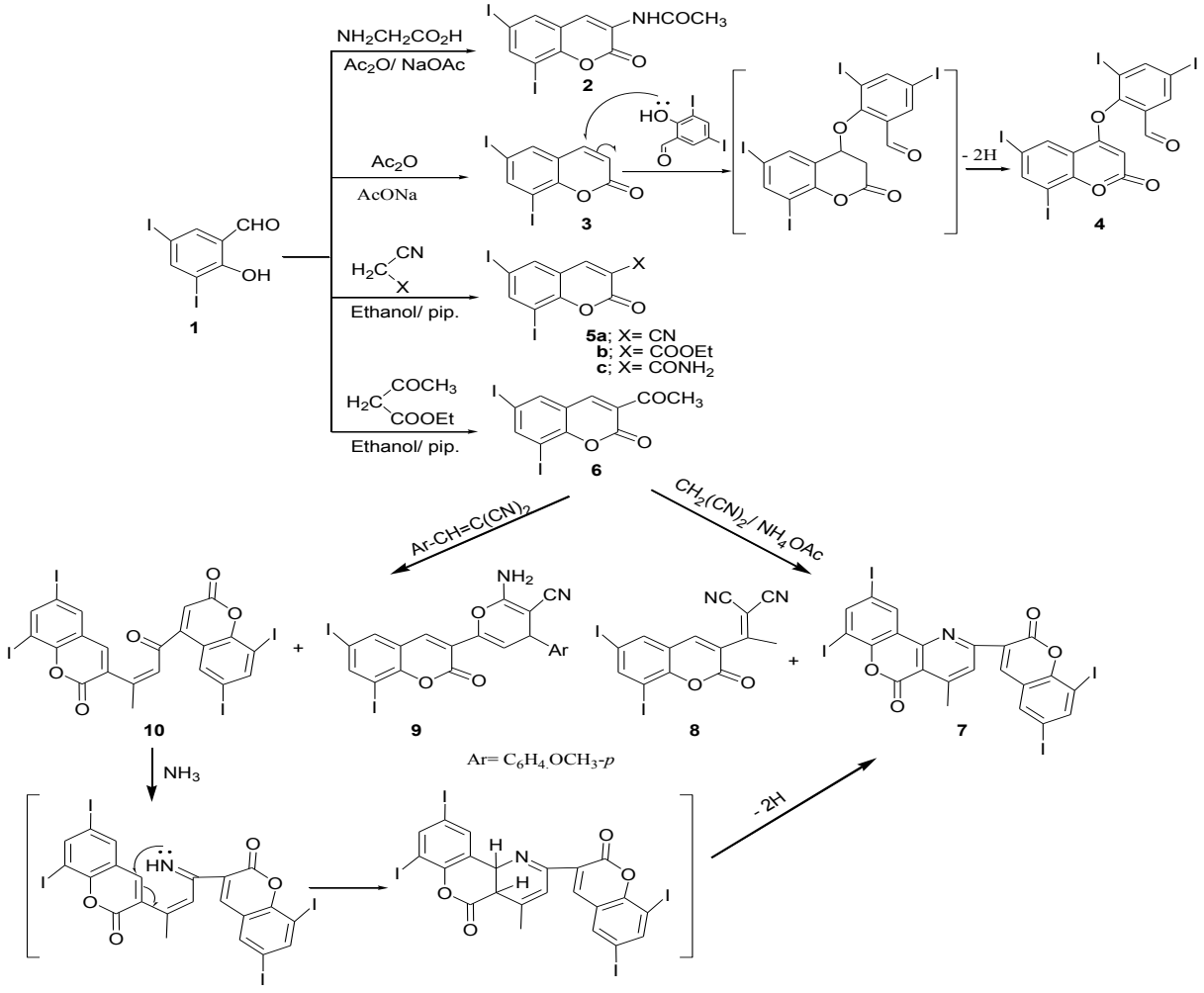
2. Results and Discussion

Condensation of 3,5-diiodosalicylaldehyde (1) with active methylene compounds afforded 6,8-diiodocurmarin derivatives (2-6). Reaction of 6 with malononitrile in boiling benzene containing NH_4OAc and few drops of AcOH afforded two reaction products, insoluble solid crystals were identified as 2-(6,8-diiodocoumarin-3-yl)-7,9-diiodo-4- methyl coumarino[4,3-*b*]pyridine (7) while the main isolated reac-

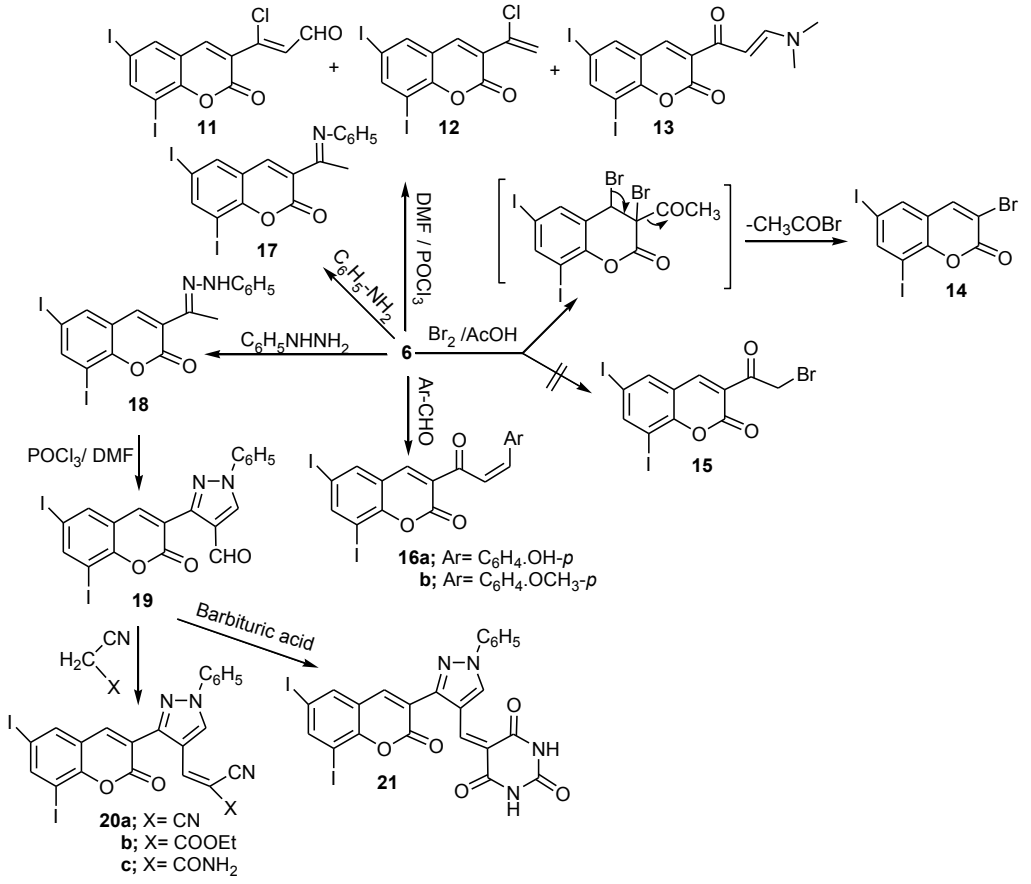
tion product from benzene was identified as 2-(1-(6,8-diiodo -coumarim-3-yl)ethylidene)malonoitrile (8). Reaction with *p*-methoxybenzylidenemalononitrile in absolute ethanol/piperidine mixture affored two reaction products pyrane derivative 9 and bis coumarin derivative 10. The formation of 9 is attributed to the addition of activated acetyl group to the activated *α, β*-unsaturated double bond by enolization then ring formation through nucleophilic addition enol hydroxyl to nitrile group, however, the formation of 10 is attributed to self-condensation of two moles of 3-acetyldiiodocoumarin under the reaction conditions to the corresponding bis coumarin derivative 10. A proposed mechanism for the formation of the by product 7 apparently results from the initial formation of bis coumarin derivative 10 and subsequent amination followed by intramolecular cyclization and oxidation (Scheme 1).

The structure of compounds 2-10 were confirmed by IR, ^{1}H NMR, ^{13}C NMR and MS. The IR spectra of compounds showed υ at 3364-3144 (NH_2), 2235-2202 (CN), 1736-1674 cm^{-1} (CO). ^{1}H NMR for compounds 5b, 5c, 6 and 8 showed δ_H at 7.73- 8.28 ppm (1H, s, H-4) and ^{13}C NMR of compound 5b showed δ 147.24 ppm (C-4), respectively. The mass spectra of compounds 2-10 gave an additional evidence for the proposed structures.

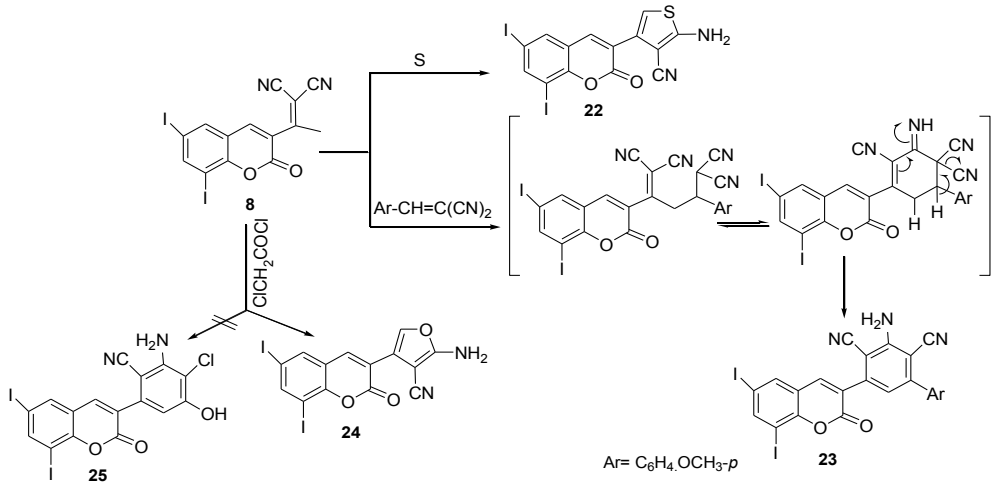
Reaction of 3-acetyl-6,8-diiodocoumarin (6) with DMF/$POCl_3$ under Vilsmeier reaction conditions[13] furnished three products, one is formulated as 3-chloro-3-(6,8-diiodocoumarin-3-yl)acrylaldehyde (11) contaminated with traces of 3-(1-chlorovinyl)-6,8-diiodocoumarin (12) and the other separated product was formulated as 3-(3-(dimethylamino)acryloyl)-6,8-diiodocoumarin (13), while bromination of 6 with Br_2/AcOH gave 3-bromo-6, 8-diiodocoumarin (14) rather than the expected *ω*-bromo derivative (15), where bromine was added to C_3-C_4 double bond, followed by loss of acetyl bromide to give 14. Treatment of 6 with aromatic aldehydes in the presence of ethanol/piperdine gave the corresponding 3-(3-arylacryloyl)-6, 8-diiodocoumarin (16a,b). While reaction of 6 with aniline or phenylhydrazine in boiling ethanol gave 6,8-diiodo-3-[1-(2-phenylhydrazono)ethyl]coumarin (17) and gave 6,8-diiodo-3-[1-(2-phenylimino)ethyl]coumarin (18), respectively. Reaction of 18 with DMF/$POCl_3$ gave 3-(6,8-diiodocoumarin-3-yl-1-phenyl-1*H*-pyrazole-4-aldehy -de (19). Condensation of 19 with some active methylene compounds to give 1-phenyl-1*H*-pyrazole derivatives (20a-c), while treatment of 19 with barbituric acid in ethanol/piperidine gave pyrimidine derivative (21) (Scheme 2).



Scheme 1. Condensation of 3,5-diiodosalicylaldehyde with active methylene compounds.



Scheme 2. Protocol synthesis of compounds 11-21



Scheme 3. Reaction of 4 with sulphur, arylidine and chloroacetyl chloride.

The structure of compounds 11-21 were established by IR and MS. The IR spectra of compounds υ at 1751-1651 cm^{-1}(CO), ^1H NMR showed δ_H 8.62-7.91 ppm (1H, s, H-4) and mass spectra of compounds 11-21 gave an additional evidence for the proposed structures. Reaction of 8 with sulphur via the Gewald reaction conditions[14] afforded thiophene derivative (22), while the reaction of 8 with *p*-methoxybenzylidenemalononitrile in absolute ethanol/piperidine gave 3-amino-5-(6,8-diiodocoumarin-3-yl) -4`-methoxybiphenyl-2,4-dinitrile (23). The formation of 23 is assumed to proceed via a Michael type addition of the methyl function in compound 8 to the activated double bond to yield the acyclic Michael adduct which then cyclized, the latter readily losses HCN to yield 23. Treatment of 8 with doublly electrophilic (chloroacetyl chloride) afforded 2-amino-4-(6,2-amino-4-(6, 8-diiodocoumarin-3-yl) furan-3-carbonitrile (24) instead of the expected 2-amino-3-chloro-6-(6,8-diiodocoumarin-3-yl)-4-hydroxy-benzonitrile (25). The formation of 24 indicates that the methyl acetyl group is transformed into a hydroxymethyl group which undergoes intramolecular cyclization to form furan ring (Scheme 3).

Table 1. Biological activity of the newly synthesized compounds.

Compd. No.[a]	Inhibition zone diameter (mm / mg sample)					
	Staphylococcus aureus (NCTC-7447)	*Bacillus cereus* (ATCC-14579)	*Escherichia coli* (NCTC-10410)	*Serratia marcescens* (IMRU-70)	*Aspergillus fumigatus* (MTCC-3008)	*Candida albicans* (MTCC-227)
2	12	9	13	10	-	-
3	10	14	10	12	-	-
4	13	10	14	15	-	-
5a	10	11	10	10	13	12
5b	10	16	14	15	12	10
5c	12	14	12	10	10	10
6	11	13	10	10	11	12
7	16	15	13	16	15	13
8	17	15	12	15	17	14
9	15	18	10	13	-	-
10	13	12	16	12	-	-
11	10	14	11	14	-	-
12	14	16	13	15	-	-
13	13	12	15	10	-	-
14	14	13	14	15	10	11
16a	10	15	10	14	10	10
16b	16	14	12	14	12	14
17	14	14	17	16	10	14
18	16	14	12	13	12	12
19	24	25	23	26	15	14
20a	25	26	24	23	17	16
20b	28	27	28	27	20	21
20c	27	28	26	27	20	20
21	26	27	25	26	19	20
22	28	28	24	28	15	18
23	24	25	27	25	21	18
24	26	27	27	20	18	17
Ampicillin	22	22	22	22	-	-
Calforan					20	20

[a] c = 1 mg ml^{-1} of new compounds in DMF.

The structure of compounds 22-24 were established by IR and MS. The IR spectra of compounds showed υ at 3355-3201 (NH_2), 2216-2191 (CN), 1735-1720 cm^{-1} (CO). The mass spectra of compounds 22-24 gave an additional evidence for the proposed structures.

The structures of all the newly synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy.

3. Antibacterial Activities

Preliminary antimicrobial screening is illustrated in Table 1. It was found that compounds 19-24 possess a pronounced antimicrobial activity against all tested microorganisms. Markedly stronger antimicrobial activity was observed against both Gram-positive and Gram-negative bacteria compared to ampicillin. Compounds 2-18 showed moderate inhibition of antimicrobial activity against both Gram-positive and Gram-negative bacteria relative to ampicillin.

Compounds 5- 8 and 14-16 showed moderate inhibition towards *Aspergillus fumigatus* and *Candida albicans* relative to Claforan. Compounds 2- 4 and 9-13 exhibited inactive inhibition compared to Claforan towards *Aspergillus fumigatus* and *Candida albicans*. Inhibition zone diameter shows a dependence on their chemical structure (Table 1).

4. Conclusions

The results from this screening demonstrated that replacing hydrogen atom attached to coumarin nucleus at C-3 with heterocyclic five membered ring the compounds 19-24 results in wide spectrum antibacterial activity against all tested Gram positive and Gram negative compared to heterocyclic six membered ring the compound 9 and the carbonocyclic ring the compound 23. Compounds possessing aliphatic side chains exhibit less antibacterial activity against all tested Gram positive and Gram negative bacteria.

5. Experimental

General

Melting points were determined on a Stuart melting point apparatus and are uncorrected. IR spectra were recorded in KBr using a FT-IR 5300 spectrometer and Perkin Elmer

spectrum RXIFT-IR system (ν, cm^{-1}). The ^1H NMR at (300 MHz) and ^{13}C NMR spectra (75MHz) were recorded in $CDCl_3$ & DMSO-d_6 on a Varian Mercury VX-300 NMR spectrometer. Chemical shifts (δ) are related to that of the solvent. Mass spectra were measured on a Shimadzu GMMS-QP-1000 EX mass spectrometer at 70eV. The elemental analyses were performed at the micro analytical center, Cairo University.

Synthesis of 3-acetylamino-6,8-diiodocoumarin (2). A mixture of compound 1 (0,37g; 10 mmol) and glycine (0.08 g; 10 mmol) in boiling glacial acetic acid (40mL), where 1gm of anhydrous sodium acetate was used as a catalyst. The crude product was obtained when the reaction was on ice. The solid obtained was filtered off and recrystallized from acetic acid Compound 2 was isolated as colourless crystals, yield 87%, 0.39 g; mp 340-342°C; IR (ν_{max}, cm^{-1}): 3332 (NH), 3078 (CH-aromatic), 1720, 1674 (CO). MS, m/z (%) = 455 (M^+, 33) and the base peak at 413 (M-CH_2=C=O, 100), 258 (M-CO-I; 24.5), 131 (M-CO-I_2; 15.7), 75 (M-2CO-I_2-CH= NH; 52). Anal. Calcd for $C_{11}H_7I_2NO_5$ (454.85): C, 29.02; H, 1.54; N, 3.08%. Found: C, 29.04; H, 1.56; N, 3.10%

Synthesis of 6,8-diiodocoumarin (3) and *2-(6,8-diiodocoumarin-4-yloxy)-3,5-diiodobenzaldehyde* (4). A mixture of compound 1 (0.37 g; 10 mmol) was dissolved in glacial acetic acid (40 mL), for which 1gm of anhydrous sodium acetate was added. The reaction mixture was refluxed for 1 hour. A solid product was precipitated while hot, which was filtered off and identified as compound 4. The filtrate was poured on a crushed ice to give solid product, which was identified as compound 3. The solid obtained was filtered off; recrystallized from ethanol/benzene and acetic acid respectively. Compound 3 was isolated as colourless; yield 35%, 0.14g; mp 205-207°C; IR (ν_{max}, cm^{-1}): 3055 (CH-aromatic), 1728 (CO). MS, *m/z* (%) = 398 (M^+, 100), 370 (M-CO; 30.5), 243 (M-CO and I; 21.6), 116 (M-CO and I_2; 28), 88 (M-2CO and I_2; 44.8). Anal. Calcd for $C_9H_4I_2O_2$ (397.83): C, 27.15; H, 1.00%. Found: C, 27.13; H, 0.99%. Compound 4 was isolated as colourless; yield 59%, 0.45g; mp 223-225°C; IR (ν_{max}, cm^{-1}): 3047 (CH-aromatic), 1720 (CO). MS, *m/z* (%) = 770 (M^+, 2.6) and the base peak (768, 100), 398 (54), 370 (18.4), 321 (11.7), 243 (10.5), 177 (15.9) and 88 (17). Anal. Calcd for $C_{19}H_6I_4O_4$ (769.64): C, 24.95; H, 0.78%. Found: C, 24.97; H, 0.80%.

Synthesis of *6,8-diiodo-3-cyanocoumarin, ethyl 6,8-diiodocoumarin-3-carboxylate* and *6,8-diiodo-3-cyanocoumarin-3-carboxamide* (5a-c). A mixture of compound 1 (0.37g; 10 mmol) and malononitrile (5a), diethyl malonate (5b),or cyanoacetamide (5c), (10 mmol) in ethanol/piperidine solution was stirred for 2 hours at room temperature. The solid obtained was filtered off and recrystallized from dioxane. Compound 5a was isolated as yellow crystals, yield 82%, 0.34g; mp 255-257°C; IR (ν_{max}, cm^{-1}): 3066 (CH-aromatic), 2202 (CN), 1712 (CO). MS, *m/z* (%) = 423 (M^+, 100), 395 (M-CO; 37.5), 268 (M-CO and I; 33.5), 141 (M-CO and I; 25.7), 113 (M-2CO and I_2; 36.1), 87 (M-CO, I_2 and CN; 30.5) Anal. Calcd for $C_{10}H_3I_2NO_2$ (422.83): C, 28.38; H, 0.71; N, 3.31%. Found: C, 28.40; H, 0.72; N, 3.33%. Compound 5b was isolated as yellow crystals, 98%, 0.46g. mp 180-200°C; IR (ν_{max}, cm^{-1}): 3050 (CH aromatic), 1727, 1687 (CO). ^1H NMR (300MHz, $CDCl_3$): δ_H 1.40 (t, *J* = 7.2Hz, 3H, CH_3), 4.41 (q, *J* = 7.2Hz, 2H, CH_2), 7.87 (d, *J* = 1.8Hz, 1H, Ar-H, H-7), 8.28 (s, H-4, 1H), 8.34 (d, *J*= 2.1Hz, 1H, Ar-H, H-5). ^{13}C NMR (300 MHz, $CDCl_3$): δ 13.97 (CH_3), 61.38 (CH_2), 86.24 (C-6), 89.24 (C-8), 118.74 (C-3), 120.22 (C-4a), 138.16 (C-5), 147.24 (C-4), 149.40 (C-7), 153.57 (C-8a), 155.07 (CO), 161.88 (CO-ester). MS, *m/z* (%) = 470 (M^+, 89), 425 (39), 398 (100), 397 (10), 370 (21), 341 (25), 214 (18), 87 (87). Anal. Calcd for $C_{12}H_8I_2O_4$ (469.85): C, 30.65; H, 1.70. Found: C, 30.66; H, 1.72%. Compound 5c was isol;ated as pale yellow crystals, yield 75%, 0.33g; mp 305-307°C; IR (ν_{max}, cm^{-1}): 3364, 3144 (NH_2), 3055 (CH-aromatic), 1750, 1726 (CO). ^1H NMR (300 MHz, $CDCl_3$): δ_H 6.0 (br, 2H, NH_2), 7.87 (d, *J* = 1.8Hz, 1H, Ar-H, H-7), 8.28 (s, H-4, 1H), 8.34 (d *J* = 2.1Hz, 1H,Ar-H, H-5). MS, *m/z* (%) = 441 (M^+, 100), 424 (M-NH_3; 17.7), 87 (14). Anal. Calcd for $C_{10}H_5I_2NO_3$ (440.84): C, 27.22; H, 1.13; N, 3.17%. Found: C, 27.24; H, 1.14; N, 3.19%.

Synthesis of 3-acetyl-6,8-diiodocoumarin (6). A mixture of compound 1 (0.37g; 10 mmol) and ethyl acetoacetate (0.13g; 10 mmol) in ethanol/ piperidine solution was refluxed for 1 hour. The solid obtained was filtered off and recrystallized from dioxane. To afford 6 as yellow crystals, yield 92%, 0.40g; mp 310-312°C; IR (ν_{max}, cm^{-1}): 3056 (CH-aromatic), 1734, 1678 (CO). ^1H NMR (300 MHz, $CDCl_3$): δ_H 2.71 (s, 3H, CH_3), 7.90 (d, *J* = 1.8 Hz, 1H,Ar-H, H-7), 8.26 (s, H-4, 1H), 8.34 (d, *J* = 2.1 Hz, 1H,Ar-H, H-5). MS, *m/z* (%) = 440 (M^+, 100), 425 (M-CH_3; 76), 398 (M-CH_2=C=O; 6.2), 397 (M-CH_3-CO; 18.8), 370 (M-CH_2=C=O; CO; 12.1), 243 (M-CH_2=C=O; CO-I; 14.4) 116 (M-CH_2=C=O, CO-I_2; 107), 87 (M-CH_2=C=O, 2CO-I_2. H; 65.1). Anal. Calcd for $C_{11}H_6I_2O_3$ (439.84): C, 30.01; H, 1.36; Found: C, 30.00; H, 1.36%.

Synthesis of 2-(6,8-diiodocoumarin-3-yl)-7,9-diiodo-4-methylcoumraino[4,3-b]pyridine (7) and *2-(1-(6,8-diiodocoumarin-3-yl)ethylidene)malononitrile* (8). A mixture of compound 6 (0.44g, 10 mmol) and malononitrile (0.66g, 10 mmol), NH_4OAc (0.07g; 10 mmol) and few drops glacial acetic acid in dry benzene (50ml) was refluxed 2 hours. A yellow solid was separated out on hot which identified compound 7, was filted off, washed with dry diethyl ether and dried. While the filtrate evaporated under reduced pressure to give compound 8; recrystallized from ethanol/benzene and dichloromethane, respectively. Compound 7 as yellow crystals, yield 25%, 0.21g; mp 214-216°C; IR (ν_{max}, cm^{-1}): 3045 (CH-aromatic), 1728 (CO). MS, *m/z* (%) = 859 (M^+, 14). Anal. Calcd for $C_{22}H_9I_2NO_4$ (858.93): C, 30.73; H,1..05; N, 1.63%. Found: C, 30.75; H, 1.07; N, 1.64%. Compound 8 as yellow crystals, yield 70%, 0.34g; mp 185-187°C; IR (ν_{max}, cm^{-1}): 3047 (CH-aromatic), 2235, 2220 (CN), 1731 (CO). ^1H NMR (300 MHz, DMSO-d_6): δ_H 2.64 (s, 3H, CH_3), 7.73 (s, 1H, H-4), 7.87 (d, *J* = 1.8Hz, 1H, Ar-H, H-7), 8.37 (d, *J* = 2.7Hz, 1H,Ar-H, H-5). MS, *m/z* (%) = 488 (M^+, 100), 461 (M –HCN; 27.7), 333 (M-HCN-HI;

15.3), 206 (M-HCN-HI-H; 72.8) and 151 (M-2HI=CO-HCN-CN; 94.3). Anal. Calcd for $C_{14}H_6I_2N_2O_2$ (488.02): C, 34.42; H,1.23; N, 5.74%. Found: C, 34.43; H, 1.25; N, 5.76%.

Synthesis of 2-amino-6-(6,8-diiodocoumarin-3-yl)- 4-(4-methoxyphenyl)-4H-pyran-3-carbonitrile (9) and *1, 3-bis(6, 8-diiodocoumarin-3-yl)but-2-enal* (10). A mixture of compound 3 (0.44g, 10 mmol), 4-methoxybenzylidine malononitrile (0.18g, 10 mmol) in absolute ethanol (20 mL), and piperidine (4 drops) was refluxed for 12 hours. A white solid precipitated on hot, to give compound 9, was filtered off washed with ethanol and dried. While the filtrate evaporated under reduced pressure to give compound 10. The solid obtained was filtered off and recrystallized from dioxane and benzene, respectively. Compound 9 as white crystals, yield 66%, 0.41g; mp 197-199°C; IR (ν_{max}, cm^{-1}): 3360, 3160 (NH_2), 3046 (CH-aromatic), 2236 (CN), 1736 (CO) MS, *m/z* (%) = 624 (M^+) together with other peaks at 581 (M-CONH), 55 (M-CONHCN). Anal. Calcd for $C_{22}H_{14}I_2N_2O_4$ (623.90): C, 42.31; H,2.24; N, 4.49%. Found: C, 42.33; H, 2.25; N, 4.51%. Compound 10 as yellow crystals 60%, 0.51g. mp 238-240°C; IR (ν_{max}, cm^{-1}): 1738, 1726 (CO) . MS, *m/z* (%) = 862(M^+, 100) , 797 (20), 541 (42), 126 (66). Anal. Calcd for $C_{22}H_{10}I_4O_5$ (861.67): C, 30.64; H,1.16%. Found: C, 30.67; H, 1.18%.

Synthesis of 3-Chloro-3-(6,8-diiodocoumarin-3-yl) acrylaldehyde (11), *3-(1-Chlorovinyl)-6,8-diiodocoumarin* (12) and *3-(3-(Dimethylamino)acryloyl)-6,8-diiocoumarin* (13). A mixture of compound 3 (0.44g, 10 mmol), DMF (20 mL) was cooled to 0 °C in an ice bath at which time $POCl_3$ (5 mL) was added dropwise on a period of 1 hour with continuous stirring. After the addition was complete, the stirring was continued for 30 min, at 0 °C and then the temperature was raised to 60 °C. The reaction mixture was stirred for 1 hour more at this temperature. The reaction mixture was then poured into cold water, acidified with HCl, and the resulting solid formed was collected by filtration, washed with (3 x 20mL), and dried to give compounds 11-13. The solid obtained was filtered off and recrystallized from benzene, chloroform and ethanol, respectively. dioxane. compound 11 as yellow crystals, yield 45%, 0.21g; .mp 140-142°C: IR (ν_{max}, cm^{-1}): 1736 (CO), 1636 (C=C). ^{1}H NMR (300MHz,$CDCl_3$): δ_H 7.65, 7.68 (d, *J* = 6.6Hz, 1H, =CH), 7.94 (d, *J* = 1.8Hz, 1H, Ar-H, H-7), 8.34 (s, 1H, H-4), 8.53, 8.36 (d, *J* = 1.8Hz, 1H, Ar-H, H-5), 10.29, 10.32 (d, *J* = 6.6Hz, 1H, CHO). Anal. Calcd for $C_{12}H_5ClI_2O_3$ (485.80): C, 29.63; H,1.04%. Found: C, 29.64; H, 1.08%. Compound 12 as yellow crystals, yield 35%, 0.15g; .mp 158-160°C: IR (ν_{max}, cm^{-1}): 1727 (CO). ^{1}H NMR (300 MHz,$CDCl_3$): δ_H 7.36 (s, 1H, $=CH_2$), 7.83 (d, *J* = 1.8Hz, 1H, Ar-H, H-7), 8.08 (s, 1H, H-4), 8.29 (d, *J* = 1.8Hz, 1H, Ar-H, H-5). Anal. Calcd for $C_{11}H_5ClI_2O_2$ (457.80): C, 28.82; H,1.10%. Found: C, 28.85; H, 1.12%. Compound 13 as yellow crystals, yield 20%, 0.09g; mp 130-132°C: IR (ν_{max}, cm^{-1}): 1734 (CO). ^{1}H NMR (300 MHz,$CDCl_3$): δ_H 3.42 (s, 6H, $N(CH_3)_2$), 5.36, 5.38 (d, *J* = 6.3Hz, 1H, =CH), 7.34, 7.36 (d, *J* = 6.3Hz, 1H, =CH), 7.85 (d, *J* = 1.8Hz, 1H, Ar-H, H-7), 8.03 (s, 1H, H-4), 8.26 (d, *J* = 1.8Hz, 1H, A-H, H-5). Anal. Calcd for $C_{14}H_{11}I_2NO_3$ (494.88): C, 33.95; H,2.22; N, 2.83%. Found: C, 33.97; H, 2.24; N, 2.85%.

Synthesis of 3-Bromo-8-ethoxycoumarin (14). A solution of compound 3 (0.44g, 10 mmol), in actic acid (10 mL), was stirred with (0.50 mL, 1.58, 10 mmol) of bromine for 2 hours in direct sun light. The solid obtained was filtered off and recrystallized from ethanol. To afford 14 as yellow crystals, yield 79% 0.32g; mp 270-272°C; IR (ν_{max}, cm^{-1}): 3055 (CH-aromatic), 1751 (CO). ^{1}H NMR (300MHz, DMSO-d_6): δ_H 7.73 (d, *J*= 1.8Hz, 1H, Ar-H, H-7), 7.91 (s, 1H, H-4), 8.28 (d, *J* = 1.8Hz, 1H, Ar-H, H-5). MS, *m/z* (%) = 476(M^+; 4.5), 475 (M-1; 50.9), 397(M-Br; 21.6), 242 (M-Br-I-CO; 17.1), 87 (M-Br-2I-2CO; 100). Anal. Calcd for $C_9H_3BrI_2O_2$ (476.83): C, 22.65; H, 0.63%. Found: C, 22.67; H, 0.64%.

Synthesis of 3-(3-(4-hydroxyphenyl)acryloyl)-6, 8-diiodocoumarin (16a) and *3-(3-(4-methoxyphenyl)acryloyl)-6, 8-diiodocoumarin* (16b) A mixture of compound 6 (0.44g; 10 mmol) and *p*-hydroxybenzaldehyde or *p*-methoxybenzaldehyde (10 mmol) was refluxed in dioxane for 6 hours after cooling. The solid obtained was filtered off and recrystallized from dioxane. Compound 16a was isolated as pale yellow crystals, yield 92%, 0.50g; mp 320-322°C; IR (ν_{max}, cm^{-1}): 3300 (OH), 3045 (CH-aromatic), 1705, 1652 (CO). MS, *m/z* (%) = 544 (M^+; 13) , 418 (9),341 (11), 262 (9), 147 (100). Anal. Calcd for $C_{18}H_{10}I_2O_4$ (544.08): C, 39.70; H,1.84%. Found: C, 39.68; H, 1.83%. Compound 16b was isolated as pale yellow crystals, yield 89%, 0.49g; mp 282-284°C; IR (ν_{max}, cm^{-1}): 3047 (CH-aromatic), 1710, 1651 (CO). MS, *m/z* (%) = 558 (M^+; 5.2), 161 (100), 577 (M-1; 42.8), 133 (55.6) and 87 (31.5). Anal. Calcd for $C_{19}H_{12}I_2O_4$ (558.11): C, 40.85; H,2.15;%. Found: C, 40.87; H, 2.17%.

Synthesis of 6, 8-diiodo-3-(1-(phenylimino)ethyl) coumarin (17) and *6, 8-diiodo-3-(1-(2-phenylhydrazono) ethyl) coumarin* (18). A mixture of compound 6 (0.44g; 10 mmol) and aniline or phenylhydrazine (10 mmol) in absolute ethanol/ DMF (1: 1; 50 ml. was refluxed for 2 hours and left to cool to room temperature. The solid obtained was filtered off and recrystallized from dioxane. Compound 17 was isolated as yellow crystals, yield 91%, 0.46g; mp 190-200°C; IR (ν_{max}, cm^{-1}): 3031 (CH-aromatic), 1720 (CO). ^{1}H NMR (300MHz, DMSO-d_6): δ_H 2.37 (s, 3H, CH_3), 7.18-7.81 (m, 5H, Ar), 7.88 (d, *J* = 1.8Hz, 1H, Ar-H, H-7), 8.50 (s, 1H,H-4), 8.32 (d, *J* = 2.7Hz, 1H, Ar-H, H-5). Anal. Calcd for $C_{17}H_{11}I_2NO_2$ (514.89): C, 39.62; H,2.14; N, 2.72%. Found: C, 39.63; H, 2.16; N, 2.73%. Compound 18 was isolated as yellow crystals, yield 91%, 0.48g; mp 205-207°C; IR (ν_{max}, cm^{-1}): 3318 (NH), 3040 (CH-aromatic), 1706 (CO). MS, *m/z* (%) = 530 (M^+; 100), 529 (33), 438 (2), 396 (6.7), 269 (2.2), 185 (20.5), 143 (8.8), 187 (38). Anal. Calcd for $C_{17}H_{12}I_2N_2O_2$ (530.10): C, 38.48; H,2.26; N, 5.28%. Found: C, 38.49; H, 2.28; N, 5.30%.

Synthesisof3-(6,8-diidocoumarin-3-yl)-1-phenyl-H-pyrazole -4-carbaldehyde (19). A mixture of compound 18 (0.53g; 10 mmol) and DMF (20 mL) was cooled to 0 °C in an ice

bath at which time $POCl_3$ (5 mL) was added dropwise on a period of 1 hour with continuous stirring. After the addition was complete, the stirring was continued for 30 min, at 0 °C and then the temperature was raised to 60 °C. The reaction mixture was stirred for 1 hour more at this temperature. The reaction mixture was then poured into cold water, acidified with HCl, and the resulting solid formed was collected by filtration, washed with (3 x 20 mL), and dried. The solid obtained was filtered off and recrystallized from dioxane. To afford 19 as yellow crystals, yield 73%, 0,41g; mp 280-282°C; IR (ν_{max}, cm^{-1}): 3036 (CH-aromatic), 1740, 1676 (CO). MS, *m/z* (%) = 568 (M^+; 19.7), 540 (100), 512 (12), 491 (9.5), 385 (6), 357 (2.7), 258 (7.8), 77 (46). Anal. Calcd for $C_{19}H_{10}I_2N_2O_3$ (568.10): C, 40.13; H,1.76; N, 4.93%. Found: C, 40.14; H, 1.78; N, 4.94%.

Synthesis of 2- ((3-(6,8-diiodocoumarin-3-yl)-1-pheny1 -1H-pyrazol-4-yl)methylene)malononitrile (20a), *ethyl 2-cyano-3-(3-(6,8-diiodocoumarin-3-yl)-1-phenyl-1H- pyrazol-4-yl)acrylate (20b), 2- cyano-3-(3-(6, 8-diiodocoumarin -3-yl)-1-phenyl-1H-pyrazol-4-yl)acrylamide* (20c) and *5-(3-(6,8-diiodocoumarin-3-yl)-1-phenyl-1H-pyrazol-4-yl)methyl ene)pyrimidine-2,4,6-(1H,3H,5H)trione* (21). A mixture of compound 13 (0.28g; 10 mmol) and malononitrile, ethyl cyanoacetate, cyanoacetamide or barbituric acid (10 mmol) in an ethanolic piperdine solution (40 mL) was refluxed for 30 min. The solid obtained was filtered off and recrystallized from dioxane. Compound 20a was isolated as pale yellow crystals, yield 85%, 0.52g; mp 330-332°C; IR (ν_{max}, cm^{-1}): 3044 (CH-aromatic), 2230, 2210 (CN), 1732 (CO). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 7.10-770 (m, 5H, N-C_6H_5), 7.76 (d, J= 1.8Hz, 1H, Ar-H, H-7), 8..62 (s, H-4, 1H), 8.36 (d, J= 2.7Hz, 1H, Ar-H, H-5)., 9.20 (s, 1H, Pyrazole), 11. 20 (s, 1H, CH=). MS, *m/z* (%) = 616 (M^+, 100), 590 (24), 489 (10), 77 (45). Anal. Calcd for $C_{22}H_{10}I_2N_4O_2$ (616.15): C, 42.85; H,1.62; N, 9.09%. Found: C, 42.87; H, 1.64; N, 9.10%. Compound 20b was isolated as yellow crystals, yield 82%, 0.54g. mp 305-307°C; IR (ν_{max}, cm^{-1}): 3126 (CH-aromatic), 2232 (CN), 1724, 1708 (CO). ^{1}H NMR (300MHz, DMSO-d_6): δ_H 1.42 (t, J= 7.2Hz CH_3, 3H), 4.31 (q, J= 7.2Hz, CH_2), 745-7.92 (m, 5H, N-C_6H_5), 7.24 (d, J= 1.8Hz, 1H, Ar-H, H-7), 8.31 (s, H-4, 1H), 8.41 (d, J= 2.7Hz, 1H, Ar-H, H-5)., 9.20 (s, 1H, Pyrazole), 10. 90 (s, 1H, CH=). Anal. Calcd for $C_{24}H_{15}I_2N_3O_4$ (663.20): C, 43.42; H,2.26; N, 6.33%. Found: C, 43.41; H, 2.28; N, 6.35%.

Compound 20c was isolated as pale yellow crystals, yield 87%, 0.55g; mp 315-317°C; IR (ν_{max}, cm^{-1}): 3318, 3200 (NH_2), 3120 (CH-aromatic), 2214 (CN), 1742, 1702(CO). MS, *m/z* (%) = 634 (M^+, 81.6), 434 (13.8), 280 (15.7), 176 (10.5), 77 (100). Anal. Calcd for $C_{22}H_{12}I_2N_4O_3$ (634.16): C, 41.63; H,1.89; N, 8.83%. Found: C, 41.61; H, 1.90; N, 8.84%. Compound 21 was isolated as pale yellow crystals, yield 91%, 0.61g. mp > 350°C; IR (ν_{max}, cm^{-1}): 3193 (NH), 3070 (CH-aromatic), 1735, 1666 (CO). MS, *m/z* (%) = 678 (M^+, 75), 579 (36.6), 540 (65), 77 (100). Anal. Calcd for $C_{23}H_{12}I_2N_4O_5$ (678.17): C, 40.70; H,1.77; N, 8.26%. Found: C, 40.72; H, 1.79; N, 8.25%.

Synthesis of 2-amino-4-(6, 8-diiodocoumarin-3-yl)thiophene-3-carbonitrile (22).A mixture of compound 8 (0.48g; 10 mmol) and elemental sulfur in dry benzene (5 0ml) was refluxed for 3 hours. The solid obtained was filtered off and recrystallized from dioxane. To afford 22 as yellow crystals, yield 85%, 0.44g. mp 320-322°C; IR (ν_{max}, cm^{-1}): 3325, 3201(NH_2), 3045 (CH-aromatic), 2191 (CN), 1720 (CO). MS, *m/z* (%) = 520 (M^+, 0), 492 (58), 365 (27.6), 210 (59), 138 (34) and 87 (47.6). Anal. Calcd for $C_{14}H_6I_2N_2O_2S$ (520.08): C, 32.30; H,1.15; N, 5.38%. Found: C, 32.32; H, 1.17; N, 5.40%.

Synthesis of 3-amno-5-(6,8-diiodocoumarin-3-yl)-4-methoxybiphenyl-2,4-dicarbonitrile (23). A mixture of compound 8 (0.48g; 5 mmol), *p*-methoxybenzylidene mlononitraile (0.92g; 5 mmol), and three drops of piperdine was refluxed in abosute ethanol (50 ml) for 2 hours. The solid obtained was filtered off and recrystallized from dioxane. To afford 23 as brown crystals, 92%, 0.59g. mp 310-312°C; IR (ν_{max}, cm^{-1}): 3355, 3160 (NH_2), 3047 (CH-aromatic), 2225, 2206 (CN), 1735 (CO). MS, *m/z* (%) = 645 (M^+, 9), 121 (100). Anal. Calcd for $C_{24}H_{13}I_2N_3O_3$ (645.19): C, 44.64; H,2.01; N, 6.51%. Found: C, 44.66; H, 2.00; N, 6.53%.

Synthesis of 2-amino-4-(6, -diiodocoumarin-3-yl)furan-3 -carbonitrile (24). A mixture of compound 8 (0.48g; 10 mmol) and chloroacetyl chloried in dry benzene (50 ml) was refluxed for 3 hours. The solid obtained was filtered off and recrystallized from dioxane. To afford 24 as yellow crystals, yield 82%, 0.41g; mp 290-292°C; IR (ν_{max}, cm^{-1}): 3359, 3162 (NH_2), 3045 (CH-aromatic), 2224, (CN), 1651 (CO). MS, *m/z* (%) = 504 (M^+, 64.8) , 490 (100), 363 (15), 235 (31), 208 (2), 179 (13), 152 (11), 135 (25.3) and 88 (19.8). Anal. Calcd for $C_{14}H_6I_2N_2O_3$ (504.029): C, 33.33; H,1.19; N, 5.55%. Found: C, 33.35; H, 1.20; N, 5.57%.

6. Antibacterial Activity

The new synthesized compounds were screened for their antimicrobial activities in vitro against two species of Gram-positive bacteria, *Staphylococcus aureus* (NCTC-7447), *Bacillus cereus* (ATCC-14579) and two Gram-negative bacteria, *Escherichia coli* (NCTC-10410), *Serratia marcescens* (IMRU-70); and two species of fungi, *Aspergillus* fumigatus (MTCC-3008) and *Candida albicans* (MTCC-227).

The activities of these compounds were tested using the disc diffusion method[15]. For bacteria and the paper disk diffusion method[16] for fungi. The area of zone of inhibition was measured using Ampicillin; (30 μgml^{-1}) as standard antibiotic and Calforan (30μgml^{-1}) was used as a reference antifungal. The tested compounds were dissolved in *N,N*-dimethylformamide (DMF) to give a solution of 1mg ml^{-1}. The inhibition zones were measured in millimeters at the end of an incubation period of 48 hours at 28°C. *N,N*-dimethylformamide (DMF) showed no inhibition zone. Test results are shown in Table 1.

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