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An Efficient and Facile Synthesis of Coumarin derivatives as Potent Antimicrobial agents

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Abstract: In the present investigation, a new class of coumarins *viz.* iminocyanocoumarins, cyanocoumarins and iminocoumarin carboxamides has been accomplished in good to excellent yields in short reaction time under water / methanol medium. The present protocol involving mild reaction condition, good to excellent yield and simple reaction procedure was found to be the best synthetic strategy for coumarin derivatives in view of environmentally benign consciousness. *In vitro* antimicrobial activity of all synthesized compounds has been evaluated against five strains of bacteria and two fungi and some of them showed their potent activity.
Keywords: Antimicrobial activity, Knoevenagel reaction, malononitrile, cyanoiminocoumarins.

1. Introduction

Recently, a considerable interest has been focused on coumarin derivatives due to their remarkably broad spectrum of biological activities including antibacterial[1-3], antifungal[4-7], antiinflammatory[8], anti coagulant[9,10], antitumor[11-13], antioxidant[14-16] and anti-HIV[17,18]. In addition, these compounds are used as fluorescent brightening agents and as dyes for tuning lasers[19-21]. The main classes are hydroxyl derivatives, especially 4- and 7-hydroxycoumarins; they are biologically potent precursors for synthesizing a coumarin family[22-26]. Owing to the significant features of coumarin derivatives in chemistry as well as pharmacology[27-29], their synthesis is of great interest. Consequently, focus is directed towards the development of elegant and eco-friendly protocols for their synthesis in the modern organic synthesis.

One of the most important features of modern synthetic methodology is the need to develop the novel methods with a low environmental impact. Taking into account that organic solvents are the main source of waste from synthetic work, the development of methods with diminished use of organic solvents is of high importance. Water as reaction medium offers many advantages such as abundantly available, non-hazardous, non-flammable, stable and cheap. Thus, water is close to being an ideal green solvent, preferable as alternatives in many organic reactions. Having minded with the above issues and in continuation of our research interest on the synthesis of heterocycles[30-32], we tempted to synthesize novel and pharmacologically active heterocycles. In this context, in the present paper we report the hybrids of coumarin system with nitrogen / sulphur heterocycles and their *in vitro* antimicrobial activities.

2. Materials and Methods

Perusal of literature indicates a lack of reports on the synthesis of coumarins *viz.* iminocyanocoumarins, cyanocoumarins and iminocoumarin carboxamides in a facile and efficient manner. Thus, herein we described the same as follows. We began our study with a solvent optimization wherein we observed that the yield of the compounds **1**, **4** and **5** was good to excellent in short reaction time (entry **1**, Table **1**) in compared with organic solvents viz. ethanol, acetonitrile, THF, toluene, xylene and benzene. In the case of compounds **2, 3** & **6-11,** methanol was found to be the best solvent affording good yields. With the above said optimized reaction condition, the scope of the reaction was established to synthesize cyanoiminocoumarins (**1, 4, 5**), cyanocoumarins (**2, 6, 7**) and carboxamidoiminocoumarins (**3, 8, 9**).

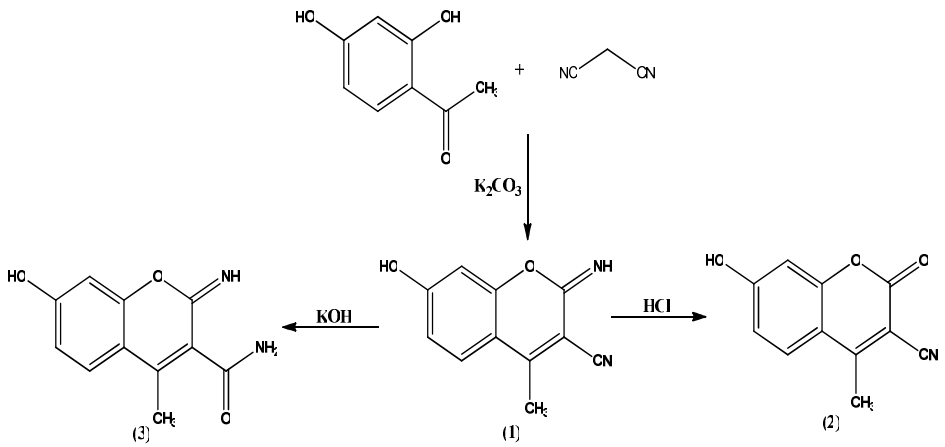
To begin with, water mediated Knoevenagel reaction was conducted between ketone and malononitrile to form a stable cyanoiminocoumarins (**1, 4, 5**) which undergoes acid hydrolysis to give cyanocoumarins (**2, 6, 7**) and base hydrolysis to afford carboxamidoiminocoumarins (**3, 8, 9**). Finally the cyanoiminocoumarins (**4**) undergoes condensation with urea and thiourea to give dipyrimidinobenzodipyrans (**10, 11**). It is mentioned that a solvent profile has carried out for the compounds (**1, 4, 5**) and we were able to isolate excellent yield with shorter reaction time (Table **1**) because the reaction was surprisingly favored in aqueous medium. Moreover, it is known that the Knoevenagel reaction is strongly solvent dependent under both homogeneous [33] and heterogeneous [34] conditions. The simplest interpretation of these results is that water brings the active methylene compound in close proximity to the negatively charged surface of the catalyst, thus favouring the ionization into carbonion donar [35]. In conclusion we report the simple procedure to prepare 3, 7-dicyano-2, 8-diimino-4, 6-dimethyl-2*H*, 8*H*-benzo[1, 2-b; 5, 4-b′]dipyran and its analogues.

3. Experimental

Melting points were determined on open capillary method and were uncorrected. The purity of the compounds was checked by TLC using silica gel-G plates and visualized by iodine vapors. IR spectra were recorded on SHIMADZU IR affinity – I spectrophotometer using pressed KBr discs. ^{1}H and ^{13}C NMR spectra were obtained on BRUKER 300 MHz spectrophotometer in DMSO (d_6) and the chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane with coupling constant (J) values in Hertz. Mass spectra were taken by SHIMADZU mass spectrometer operating at an ionization potential of 70eV. Elemental analyses for C, H, N and S were performed with Vario EL III elementer analysiser and all the compounds were in agreement with the calculated values within ±0.4%. The column chromatography was performed silicagel (60-120 mesh). In addition, reagents and solvents used were purchased from Merck Chemicals Pvt. India and used without further purification.

Synthesis of cyanoiminocoumarins (1, 4, 5)

A mixture of substituted resorcinol (1 mmol) and malononitrile (1 mmol / 2 mmol) in 15 ml of 10% potassium carbonate solution was stirred at room temperature for 15 min. The progress of the reaction was monitored by TLC. After the completion of the reaction the reaction mixture was poured into crushed ice and kept overnight in refrigerator. The solid mass thus settled was filtered off, washed several times with water and dried. After drying, the product was coloumn chromatographed with a mixture of ethylacetate: chloroform(1:3) to afford pure cyanoiminocoumarins.



Scheme 1. Synthesis of coumarins from monoacetyl resorcinol.

Synthesis of cyanocoumarins (2, 6, 7)

A dry round bottomed flask fitted with a reflux condenser was charged with 5mmol of iminocyanocoumarin **(1/ 4/ 5)** and 25 ml of methanol. After complete dissolution, con. HCl (0.5 ml) was added and refluxed for 30min. After the completion of the reaction the content was allowed to cool at room temperature for an hour. After the completion of the reaction, the solvent was removed. The solid separated out was filtered, dried and recrystallised from ethanol to give cyanocoumarins.

Synthesis of carboxamido iminocoumarins (3, 8, 9)

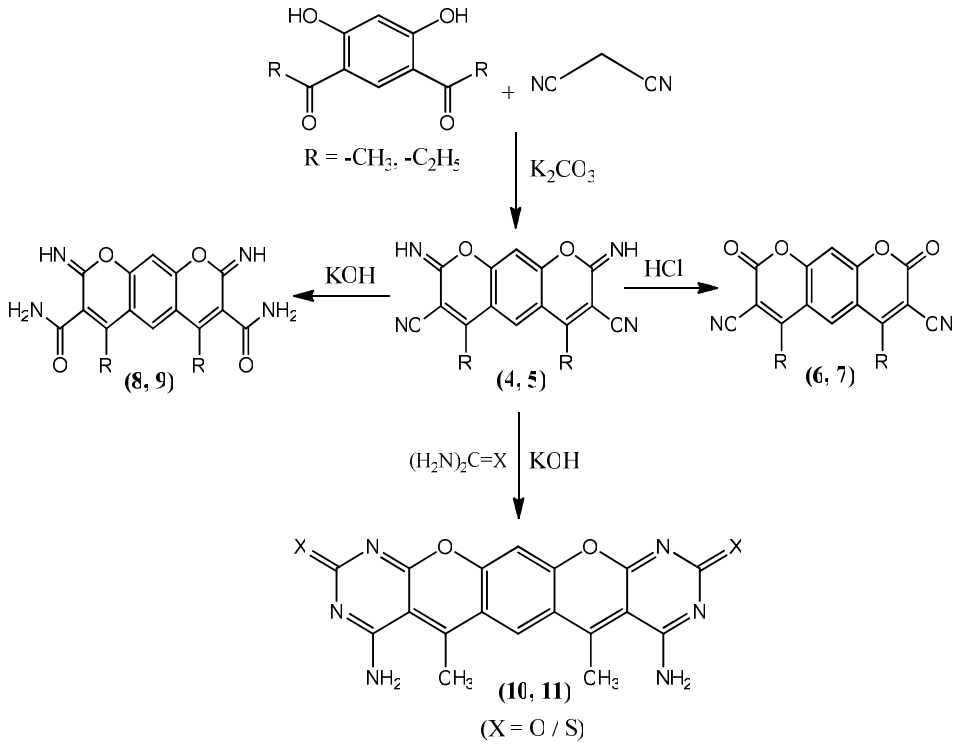
To a solution of cyanoiminocoumarin (1mmol) dissolved in 25 ml of methanol, 2 ml of 10% aqueous KOH was added. Then the content was refluxed on water bath for 6 h. After the completion of the reaction the content was allowed to cool at room temperature for about an hour. The reaction mixture was poured into excess of crushed ice and neutralized with dil. HCl. The residue thus obtained was washed several times with water and recrystallized from methanol. The purity of the synthesized compounds was checked by TLC.

Synthesis of 5, 9-diamino-6, 8-dimethyl-3, 11-dioxo / dithieno-3, 11-dihydrodipyrimidino [4, 5-b; 5, 4-e]benzo [1, 2-b; 5, 4-b']dipyran (10 / 11)

A mixture of compound **4** (1 mmol) and urea / thiourea (2 mmol) and 2 ml of 10% aqueous solution of KOH in 25 ml of methanol was taken in a round bottomed flask. The content was refluxed on water bath for 8 h. Then the reaction mixture was poured into excess of crushed ice and neutralized with dil. HCl. The residue thus obtained was washed several times with water and recrystallized from ethanol. The purity of the synthesized compounds was checked by TLC.

4. Results and Disscusion

The structure of all the synthesized compounds was assigned by IR, mass, NMR (^{1}H & ^{13}C) spectra and elemental analysis. The ^{1}H NMR spectrum of **1** exhibited a sharp singlet at δ 2.26 ppm which is assigned for the protons of CH_3 group, a singlet at δ 8.36 ppm which is assigned to imino proton and two doublets, one singlet in the range of δ 7.17 to 7.70 ppm are owing to aromatic protons. The ^{13}C NMR spectrum of **1** showed a signal at 15.89 ppm for $\underline{C}H_3$ carbon and another signal at 117.84 ppm due to $\underline{C}\equiv N$. The mass spectrum of **1** showed molecular ion peak at m/z = 200(M^+). The IR spectrum of compound **1** exhibited OH stretching frequency at 3446 cm^{-1}, NH and C≡N stretching frequencies at 3313 and 2210 cm^{-1} respectively. The compound **2** exhibited a band at 1734cm^{-1} due to C=O of coumarin moiety. The characteristic frequencies have shown for each compound of the series.



Scheme 2. Synthesis of coumarins from diacetyl / dipropanoyl resorcinol.

Table 1. Optimization for the synthesis of cyanoiminocoumarins (**1**, **4, 5**).

Entry	Solvent	Time (min)	Yield (%)		
			1	**4**	**5**
1	Water	15	85	92	88
2	Ethanol	30	65	80	74
3	Acetonitrile	45	55	68	60
4	THF	90	30	33	24
5	Toluene	180	15	16	10
6	Xylene	220	12	18	10
7	Benzene	240	12	18	12

3-Cyano-7-hydroxy-2-imino-4-methyl -2*H*-benzo[1, 2-b]pyran 1

Yield: 65% mp: 170 ^{o}C, orange solid, Anal. Calcd. for $C_{11}H_8N_2O_2$: C, 66.00; H, 4.03; N,13.99 Found: C, 65.96; H, 4.00; N, 13.95; **IR(υ cm^{-1}) KB**r: 3446(OH), 3313(NH), 2210(C≡N), 1648(C=N), **^{1}H NMR (DMSO d$_6$):** δ 2.26 (3H, s, CH_3), 7.17(1H, d , J=8.1Hz, C_6-H), 7.30 (1H, s, C_8-H), 7.70 (1H, d, J=7.8Hz, C_5-H), 8.36(1H, s, =NH), 10.42 (1H, s, -OH), **^{13}C NMR (DMSO d$_6$):** δ 15.89(CH_3), 81.88(C_3), 99.12(C_8), 117.04(C≡N), 126.15(C_5), 127.84(C_6),129.04(C_a), 153.09(C_4) 155.91(C_7), 156.27(C_2), **m/z:** 200(M^+, 100%), 186(6%), 134(9%), 118(16%).

3-Cyano-7-hydroxy-4-methyl-2-oxo-2*H*-benzo[1, 2-b]pyran 2

Yield: 68% mp: 210 ^{o}C, yellow solid, Anal. Calcd. for $C_{11}H_7NO_3$: C, 65.67; H, 3.51; N, 6.96, Found: C, 65.69; H, 3.47; N, 7.00, **IR(υ cm^{-1}) KB**r: 3448(OH), 3062(C-H_{Ar}), 2233(C≡N), 1734(C=O, lactone), 1625(C=N), **^{1}H NMR(DMSO d$_6$):** δ 2.10 (3H, s, CH_3), 7.17(1H, d, J=7.5Hz, C_6-H), 7.51(1H, s, C_8-H), 7.92(1H, d, J=7.5Hz, C_5-H), 10.70(1H, s, OH), **^{13}C NMR (DMSO d$_6$):** δ 13.82(CH_3), 88.63(C_3, C_8), 103.97 (C_6, C_b), 118.35(C≡N), 128.39(C_5), 136.10(C_a), 149.57(C_4, C_7), 157.98(C_2), **m/z:** 202($M+H^+$, 100%), 176(88%), 162(7%), 110(22%).

3-Carboxamido-7-hydroxy-2-imino-4-methyl-2*H*-benzo[1, 2-b]pyran 3
Yield: 62% mp: 142 °C, pale yellow solid, Anal. Calcd. for $C_{11}H_{10}N_2O_3$: C, 60.55; H, 4.62; N, 12.84, Found: C, 60.52; H, 4.65; N, 12.83, **IR(υ cm^{-1}) KB**r: 3421(OH), 3207, 3321(NH, NH_2), 3041 (C-H_{Ar}), 1658(C=O), 1585 (C=C), 1618(C=N), **^{1}H NMR (DMSO d_6):** δ 2.18 (3H, s, CH_3), 7.04(1H, d, J=9.0Hz, C_6-H), 7.74(1H, d, J=8.7Hz, C_5-H), 8.05 (1H, s, C_8-H), 8.49(2H, br, s, NH_2), 8.86(1H, s, NH), 11.61(1H, s, OH), **^{13}C NMR (DMSO d_6):** δ 11.62(CH_3), 68.83(C_3), 95.82(C_8), 102.12(C_6, C_b), 120.80 (C_5), 134.30(C_a), 160.93(C_4, C_7), 162.42(C_2), **m /z:** 218(M^+, 12%), 147(100%), 204(22%), 176(16%), 118(28%), 104(33%).

3, 7-Dicyano-2, 8-diimino-4, 6-dimethyl-2*H*, 8*H* -benzo[1,2-b; 5,4-b′]dipyran 4
Yield: 80% mp: 214 °C, orange solid, Anal. Calcd. for $C_{16}H_{10}N_4O_2$: C, 66.20; H, 3.47; N,9.57 Found: C, 66.22; H, 3.45; N, 10.00, **IR(υ cm^{-1}) KB**r: 3317(NH), 2214(C≡N), 1604(C=N), 1175 (C-O-C), **^{1}H NMR (DMSO d_6):** δ 2.25 (6H, s, 2 -CH_3), 6.36 (1H, s, C_5-H), 7.76 (1H, s, C_{10} -H), 8.40(2H, s, 2 -NH), **^{13}C NMR (DMSO d_6):** δ 12.46 (C_4, C_6-CH_3), 102.50(C_3, C_7), 110.91(C_{10}), 114.20(C≡N), 125.60(C_5), 130.55(C_b), 152.37(C_a), 159.79(C_2, C_8), 162.25(C_4, C_6), **m /z:** 291(M +H^+,15%), 241(100%), 240(20%), 215(11%), 199(8%), 145(10%), 118 (8%).

3, 7-Dicyano-2, 8-diimino-4, 6-diethyl-2*H*, 8*H* -benzo[1,2-b; 5,4-b′]dipyran 5
Yield: 74%, mp: 278 °C, red solid, Anal. Calcd. for $C_{18}H_{14}N_4O_2$: C, 67.91; H, 4.43; N,17.60; Found: C, 67.89; H, 4.40; N, 17.59, **IR(υ cm^{-1}) KB**r: 3278(=NH), 2927(C-H_{aliph}), 2220(C≡N), 1568(C=N), 1232 (C-O-C), **^{1}H NMR (DMSO d_6):** δ 1.18 (6H, t, J=7.2Hz, 2 -CH_3),3.01 (4H, q, J=7.5Hz, 2 -CH_2) 6.69(1H, s, C_5-H), 8.11(1H, s, C_{10} -H), 8.77(2H, s, 2 =NH), **^{13}C NMR (DMSO d_6):** δ 14.04(C_4, C_6-CH_3), 33.19($\underline{C}H_2$), 84.41(C_3), 104.79(C_{10}), 107.80(C_6), 116.43 ($\underline{C}$≡N), 129.71(C_5), 157.42(C_a), 170.03($\underline{C}$=NH), **m/z:** 319(M+H^+, 32%), 268(100%), 214(18%), 157(45%), 118(10%), 100(17%).

3, 7-Dicyano-4, 6-dimethyl-2, 8-dioxo-2*H*, 8*H*-benzo[1,2-b; 5,4-b′]dipyran 6
Yield: 64% mp: 146 °C, yellow solid, Anal. Calcd. for $C_{16}H_8N_2O_4$: C, 65.76; H, 2.76; N, 9.59 Found: C, 65.78; H, 2.75; N, 9.57, **IR(υ cm^{-1}) KB**r: 3062(C-H_{Ar}), 2924(CH_3), 2233(C≡N), 1735(C=O), **^{1}H NMR (DMSO d_6):** δ 2.63 (6H, s, 2 -CH_3), 6.57(1H, s, C_5-H), 8.15 (1H, s, C_{10}-H), **^{13}C NMR (DMSO d_6):** 17.82(C_4, C_6-CH_3), 104.31(C_3, C_7), 109.28(C_{10}), 116.19(C≡N), 129.82(C_5), 150.29(C_b), 155.62(C_a), 162.72(C_2, C_8), 168.81(C_4, C_6), **m/z:** 293 (M+H^+, 43%), 104(100%), 267(14%), 243(68%), 187(22%), 158(18%), 149(35%), 118(15%).

3, 7-dicyano-4, 6-diethyl-2, 8-dioxo-2*H*, 8*H* -benzo[1,2-b; 5,4-b′]dipyran 7
Yield: 55% mp: 320 °C, yellow solid**,** Anal. Calcd. for $C_{18}H_{12}N_2O_4$: C, 67.50; H, 3.78; N, 8.75; Found: C, 67.49; H, 3.80; N, 8.73; **IR(υ cm^{-1}) KB**r: 3080(C-H_{Ar}), 2926(CH_3), 2206(C≡N), 1735(C=O), 1653(C=N), 1369 (C-O-C), **^{1}H NMR (DMSO d_6):** δ 1.24 (6H, t, J=7.5Hz, 2 -CH_3), 3.14 (4H, q, J=7.2Hz, 2 -CH_2) 6.88(1H, s, C_5-H), 8.25(1H, s, C_{10}-H), **^{13}C NMR (DMSO d_6):** δ 12.08(C_4 , C_6-CH_3), 27.91($\underline{C}H_2$), 81.84(C_3), 103.21(C_{10}), 108.45(C_b), 117.93 ($\underline{C}$≡N), 124.28 (C_5), 161.91(C_a), 164.03($\underline{C}$=NH), **m/z:** 321 (M+H^+, 30%), 110(100%), 270(10%), 242(12%), 214(9%), 158(20%).

3, 7-Dicarboxamido-4, 6-dimethyl-2, 8-diimino -2*H*, 8*H* -benzo[1,2-b; 5,4-b′]dipyran 8
Yield: 52% mp: 167 °C, pale yellow solid, Anal. Calcd. for $C_{16}H_{14}N_4O_4$: C, 58.89; H, 4.32; N,17.17 Found: C, 58.86; H, 4.36; N, 17.19, **IR(υ cm^{-1}) KB**r: 3325, 3290(NH_2), 3155(=NH_2), 1665(C=O), 1598(C=N), 1544(C=C), 1354(C-O-C), 1228(C-N), **^{1}H NMR (DMSO d_6):** δ2.37(6H, s, 2 -CH_3), 6.28(1H, s, C_5-H), 7.37(1H, s, C_3-H), 7.76(4H, s, 2 -NH_2), **^{13}C NMR (DMSO d_6):** δ 12.21(C_4, C_6-CH_3), 103.78(C_3, C_7), 112.68(C_{10}), 128.04(C_5), 145.65(C_b), 149.94(C_a), 163.54(C=O), **m /z:** 327(M^+, 23%), 242(100%), 294(12%), 214(12%), 188(21%).

3, 7-Dicarboxamido-4, 6-diethyl-2, 8-diimino-2*H*, 8*H* -benzo[1,2-b; 5,4-b′]dipyran 9
Yield: 60% mp: 210 °C, yellow solid, Anal. Calcd. for $C_{18}H_{18}N_4O_4$: C, 58.89; H, 4.32; N,17.17 Found: C, 58.90; H, 4.36; N, 17.18, **IR(υ cm^{-1}) KB**r: 3383, 3336(NH_2), 3128(NH), 2975(CH_3), 1645(C=O), 1645(C=N), 1365 (C-O-C), **^{1}H NMR (DMSO d_6)**: δ 1.26(6H, t, J=5.4Hz, 2 -CH_3), 3.00(4H, q, J=7.2Hz, 2 -CH_2) 6.44(1H, s, C_5-H), 7.89(4H, s, 2 -NH_2), 8.29(1H, s, C_{10}-H), 8.90(2H, s, 2 =NH), **^{13}C NMR (DMSO d_6):** δ 13.41(C_4 , C_6-CH_3), 28.28($\underline{C}H_2$), 79.21(C_3), 101.26(C_{10}), 107.21(C_b), 128.04(C_5), 138.03(C_a), 149.93($\underline{C}$=NH), 163.52(C=O), **m/z:** 355(M+H^+, 22%), 268(100%), 214(21%), 158(13%), 110(18%).

5, 9-Diamino-6, 8-dimethyl-3, 11-dioxo-3, 11-dihydrodipyrimidino[4, 5-b; 5, 4-e]benzo[1, 2-b ; 5, 4-b']dipyran 10

Yield: 62% mp: 185 ^{o}C, yellow solid**,** Anal. Calcd. for $C_{18}H_{12}N_6O_4$: C, 57.45; H, 3.21; N, 22.33 Found: C, 57.48; H, 3.22; N, 22.31, **IR(υ cm^{-1}) KB**r: 3394, 3325(-NH_2), 3057(C-H_{Ar}), 2920(-CH_3), 1739(C=O), 1653(C=N), **^{1}H NMR (DMSO d$_6$):** δ 2.70 (6H, s, 2 -CH_3), 6.90(4H, s, 2 -NH_2), 6.96(1H, s, C_5-H), 8.30 (1H, s, C_{10}-H). **^{13}C NMR (DMSO d$_6$):** δ18.09(C_4, C_6-CH_3), 105.76(C_3, C_7), 106.41(C_b), 111.06(C_{10}), 120.17(C_5), 137.47(C-NH_2), 157.07(C_a), 163.01(C_2, C_8), 165.41(C_4, C_6)179.98(C=O), **m/z:** 376(M^+, 18%), 266(100%), 335(8%), 281(22%), 242(20%), 160(9%), 130(11%), 109(6%).

5, 9-Diamino-6, 8-dimethyl-3, 11-dithieno-3, 11-dihydrodipyrimidino[4, 5-b; 5, 4-e]benzo[1, 2-b ; 5, 4-b'] dipyran 11

Yield: 77% mp: 140 ^{o}C, brown solid, Anal. Calcd. for $C_{18}H_{12}N_6O_2S_2$: C, 52.93; H, 2.96; N, 20.58; S,15.70 Found: C, 52.94; H, 2.99; N, 20.56; S, 15.71, **IR(υ cm^{-1}) KB**r: 3399, 3331(-NH_2), 3080(C-H_{Ar}), 2926(-CH_3), 1257(C=S), 1653(C=N), **^{1}H NMR (DMSO d$_6$):** δ 2.0(6H, s, 2 -CH_3), 6.42(4H, s, 2-NH_2), 6.84(1H, s, C_5-H), 7.58(1H, s, C_{10}-H), **^{13}C NMR (DMSO d$_6$):** δ 15.16 (C_4, C_6, -CH_3), 103.21(C_3, C_7), 107.39(C_b), 114.52(C_{10}), 127.91(C_5), 130.44 (C-NH_2), 139.62(C_a), 157.28(C_2, C_8), 160.48(C=S), **m/z:** 408(M^+, 28%), 102(100%), 368(10%), 299(9%), 282(35%), 266(10%), 243(15%), 158(6%), 142(16%), 130(18%), 118(6%).

Antimicrobial Activity

Antibacterial activity: The purified products were screened for their antibacterial activity by well diffusion method using ciprofloxacin as a standard antibiotic drug. The nutrient agar prepared by the usual method, was inoculated aseptically with 0.5 ml of overnight subculture of *Escherichia coli*, *Proteus sp*., *Vibrio cholerae*, *Pseudomonas aeruginosa and Salmonella typhi* in separate conical flasks at 30 ºC and mixed well by gentle shaking. About 25 ml of the contents of the flasks were poured and evenly spread in petridish (90 mm in diameter) and allowed to set for 2 h. Mueller Hinton agar (beef infusion solids 4.0 g, starch 1.5 g, casein hydrolysate 17.5 g, agar 15.0 g, final pH 7.4 ± 0.2 at 37 °C) was used for antimicrobial assay. The assay plates were prepared by spread plate technique with appropriate pathogen inoculums (~10^4 CFU). Using a sterile cork borer, a 7 mm well was made and filled with 0.05 ml (50 µg/ml of solution of sample in DMSO). The plates were incubated at 37 ºC for 24 h and the control was also maintained with 0.05 ml of DMSO in similar manner and the zones of inhibition of the bacterial growth were measured in millimeter and recorded in Table **2**. Compound **4** and **11** have found to possess appreciable activity against all the organisms.

Antifungal activity: *Aspergillus niger* and *Candida albicans* were used for testing antifungal activity by well diffusion method. The culture was maintained on Sabouraud dextrose agar (SDA) slants. SDA medium was inoculated with 72 h old 0.5 ml suspension of fungal spores in a separate flask. About 25 ml of the inoculated medium was evenly spread in a sterilized petridish and allowed to set for 2 h. The well (4 mm in diameter) were punched in petridish and loaded with 0.05 ml (10 µg/ml) of solution of sample in DMSO. The plates were incubated at 30 ^{o}C for 4-8 hours after the completion of incubation period, the zones of inhibition of growth in the form of diameter in mm was measured. Along the test solution in each petridish on well was filled up with solvent which acts as control. The zones of inhibition are recorded in Table **2**. Compound **4** and **11** have found to posses excellent activity against *C. albicans and A. niger* and compound 3 has shown no remarkable effect against the chosen organisms.

5. Conclusion

In conclusion, a facile and efficient synthesis of a new class of coumarins *viz.*, iminocyanocoumarins, cyanocoumarins and iminocoumarin carboxamides has been achieved in water medium/methanol via Knoevenagel reaction. Our proposed protocol involving water as a reaction medium, mild reaction condition, good to excellent yield and simple work-up procedure was found to be the best in view of environmentally benign consciousness. *In vitro* antimicrobial activity of all synthesized compounds has been evaluated against five strains of bacteria and two fungi and some of them showed their potent activity.

Table 2. Antibacterial and antifungal activity of the coumarin derivatives (1-11).

Antibacterial activity zones of inhibition (mm)* Std: **Ciprofloxacin** (50 μg/ml)						Antifungal activity zones of inhibition(mm)* Std:**Nystatin**(10 μg/ml)	
Compound	***E.coli***	***Proteus sp.***	***Vibrio cholerae***	***P.aeruginosa***	***Salmonella typhi***	***A. niger***	***C.albicans***
1	18	11	18	13	14	13	18
2	14	09	12	10	09	14	12
3	20	-	08	12	07	-	-
4	30	17	23	13	22	23	21
5	18	10	09	07	16	12	10
6	15	08	07	09	11	15	13
7	13	07	18	-	10	-	20
8	21	-	13	06	09	13	12
9	19	-	11	10	13	15	11
10	20	10	21	14	14	19	16
11	30	14	24	16	15	22	20
+ve Control	24	16	20	22	18	30	25
-ve Control	-	-	-	-	-	-	-

*zone of inhibition is obtained as a mean value of triplicates
+ve Control: Standard Ciprofloxacin / Nystatin was used as positive control dissolved in DMSO. -ve Control: Solvent DMSO alone was used to study the physical inhibition.

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