Journal of the Korean Chemical Society
2008, *Vol. 52, No. 3*
Printed in the Republic of Korea

새로운 Tetrazole유도체의 합성과 항균활성

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(2007. 10. 16 접수)

Synthesis and Antibacterial Activity of New Tetrazole Derivatives

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(Received October 16, 2007)

요 약. 3-Acetyl/Formyl 4-hydroxy-2*H*(1)-benzopyran-2-one를 malonitrile와 ethyl cyanoacetate로 처리하여 각각 1,1-dicyano-2-[4′-hydroxy-2′*H*(1)-benzopyran-2′-one-3′-yl] ethene/propene **2a-h**와 ethyl-2-cyano-3-[4′-hydroxy-2′*H* (1)-benzopyran-2′-one-3′-yl] propenoate/butenoate **3a-h**를 얻었다. NaN_3와 **2a-h**의 1.3 dipolar 반응에서 **4a-h**인 tetrazole유도체를 얻었다. **3a-h**는 PPA를 이용한 고리화 반응으로 3-cyano-2*H*,5*H*-pyrano [3, 2-*c*] benzopyran-2,5-diones **5a-h**를 얻었다. **5a-h**는 NaN_3와1.3 dipolar 반응으로 -(1′*H*-tetrazol-5′-yl)-2*H*,5*H*-pyrano[3, 2-*c*] benzopyran-2,5-diones **6a-h**얻었다. 화합물의 구조는 스펙트럼과 자료 분석을 기초로 입증했다.모든 화합물은 항균 활성을 검사하였고 의미있는 항균성을 가짐을 밝혔다. **2h**화합물과 **4h** 화합물은 50 μg/mL에서 활성을 보였다

주제어: 4-하이드록시 쿠마린, knoevenegal 축합반응, 1.3 dipolar 반응, 항균활성

ABSTRACT: 3-Acetyl/Formyl 4-hydroxy-2*H*(1)-benzopyran-2-one on treatment with malonitrile and ethyl cyanoacetate yielded 1,1-dicyano-2-[4′-hydroxy-2′*H*(1)-benzopyran-2′-one-3′-yl] ethene/propene 2a-h and ethyl-2-cyano-3-[4′-hydroxy-2′*H* (1)-benzopyran-2′-one-3′-yl] propenoate/butenoate 3a-h respectively. The 1,3 dipolar reaction of 2a-h with NaN_3 gave the tetrazole derivative 4a-h. 3a-h on cyclization with PPA gave 3-cyano-2*H*,5*H*-pyrano [3, 2-*c*] benzopyran-2,5-diones 5a-h which on 1,3 dipolar reaction with NaN_3 to gave 3-(1′*H*-tetrazol-5′-yl)-2*H*,5*H*-pyrano[3, 2-*c*] benzopyran-2,5-diones 6a-h. The structures of the compounds have been established on the basis of the spectral and analytical data. All the compounds were screened for their antimicrobial activities and have been found to exhibited significant antibacterial activities. Compounds 2h and 4h showed the activity 50μg/mL.

Keywords: 4-hydroxy Coumarin, Knoevenegal Condensation, 1,3 Dipolar Reaction, Antibacterial Activity

INTRODUCTION

Benzopyran-2-ones and pyranobenzopyrones are well known for their biological activities.[1-3] Tetrazoles and their derivatives are reputed CNS drugs and having wide application as sedatives[4] and antihypertensive drugs. They are also known for antiallergic,[4] antimicrobial,[5] antilipemic,[6,7] carboxylic acid isosteres,[8] anticholinergic, antiinflammatory,[9] hormonal[10] and diuretics[11] activity. They are used as herbicides[12] and radio protective agents.[10] By observing these biological properties, it was thought of synthesizing tetrazole moiety, which is either attached to coumarin or pyranobenzopyran moiety, which may have the above biological activity. All the synthesized compounds were screened for their antibacterial activity.

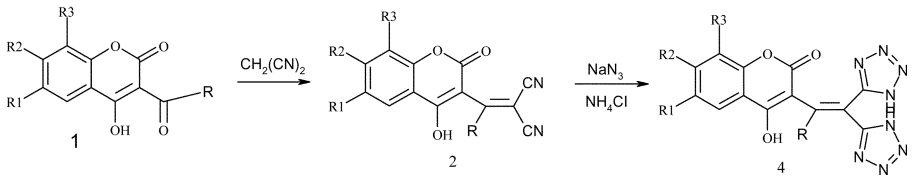
RESULTS AND DISCUSSION

3-Acetyl-4-hydroxy-2*H* (1)-benzopyran-2-one[13] **1a-d** and 3-formyl-4-hydroxy-2*H*(1)-benzopyran-2-one[14] **1e-h** were treated with compounds having active methylene group such as malonitrile and ethyl cyanoacetate in presence of piperidine to undergo Knoevenegal condensation to yield 1,1-dicyano-2-[4′-hydroxy-2′*H*(1)-benzopyran-2′-one-3′-yl]ethene/propene **2a-h** and ethyl-2-cyano-3-[4′-hydroxy-2′*H*(1)-benzopyran-2′-one-3′-yl]propenoate / butenoate **3a-h**. **2a-h** further undergoes 1,3 dipolar reaction with NaN_3 to give 1,1-di (1*H* tetrazol-5-yl)-2-[4′-hydroxy-2′*H*(1)-benzopyran-2′-one-3′-yl]-ethene/propene **4a-h**. **3a-h** on cyclization with PPA gave 3-cyano-2*H*,5*H*-pyrano[3,2-*c*]benzopyran-2,5-dione **5a-d** and 3-cyano-4-methyl-2*H*,5*H*-pyrano [3,2-*c*]benzopyran-2,5-dione **5e-h**. 1,3 dipolar reaction of **5a-h** with NaN_3 gave 3-(1′*H*-tetrazol-5′-yl)-2*H*,5*H*-pyrano[3,2-*c*]benzopyran-2,5-dione **6a-d** and 4-methyl-3-(1′*H*-tetrazol-5′-yl)-2*H*, 5*H*-pyrano [3,2-*c*]benzopyran-2,5-dione **6e-h**. The structures of the compounds **2a-h** to **6a-h** were confirmed on the basis of spectral and analytical data. All the above compounds were screened for their antimicrobial activities.

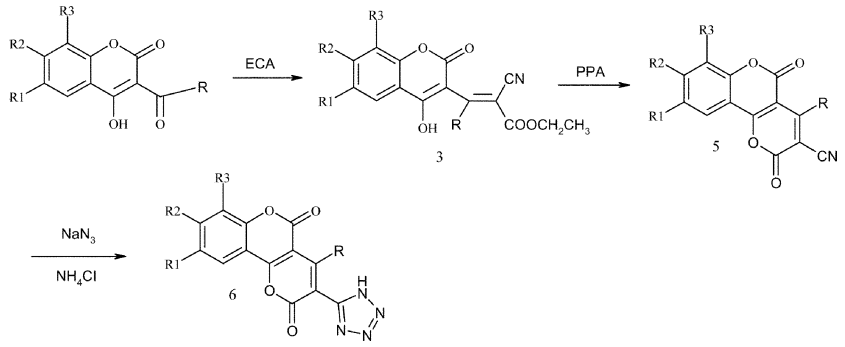
The minimum inhibition concentration (MIC) was determined using Tube Dilution method according to standard procedure.[15] All the compounds were screened in vitro for their antimicrobial activity against variety of bacterial strains such as *Staphylococcus aureus*, *Salmonella para-typhi* and *Escherichia coli* (*Table* 1). The standard drugs used for comparison were ciprofloxacin (MIC 5 μg/mL), cloxacillin (MIC 10 μg/mL) and gentamycin (MIC 5 μg/mL).

CONCLUSION

The antibacterial activity of the compounds 2-6(a-h) was compared and it was found that amongst them compound 2h and 4h showed significant activity against *S.aureus*. *S.typhi* and *E.coli*. While compounds 2b, 2d, 3h, 4d, 5d, 5h and 6h showed average activity. Compounds 2h and 4h, which lack the pyranonebenzopyranone fused ring system and



Scheme 1



1-6a: R_1, R_2, R_3=H, R=H
1-6b: R_1=CH_3, R_2, R_3=H, R=H
1-6c: R_1, R_3=H, R_2=CH_3, R=H
1-6d: R_1, R_2=H, R_3=CH_3, R=H
1-6e: R_1, R_2, R_3=H, R=CH_3
1-6f: R_1=CH_3, R_2, R_3=H, R=CH_3
1-6g: R_1, R_3=H, R_2=CH_3, R=CH_3
1-6h: R_1, R_2=H, R_3=CH_3, R=CH_3

Scheme 2

Table 1. Antimicrobial activity data (MIC μg/mL) of compounds **2-6.**

Compound	Antibacterial μg/mL			Compound	Antibacterial μg/mL		
	S.aureus	*S.typhi*	*E.coli*		*S.aureus*	*S.typhi*	*E.coli*
2a	-	+	-	**4e**	++	-	-
2b	+++	++	++++	**4f**	+++	++	++
2c	+	+	+	**4g**	+	+	+++
2d	++++	+++	+++	**4h**	+++++	+++	++
2e	-	-	++	**5a**	+++	++	+
2f	++	++	++	**5b**	+	+	+++
2g	++	++	-	**5c**	+	+	+++
2h	+++++	-	+++	**5d**	++++	++++	++
3a	++	+++	+++	**5e**	-	++	+++
3b	-	-	++	**5f**	++	++	+++
3c	+	+	++	**5g**	+	+	++
3d	+++	+	++	**5h**	+++	++++	+++
3e	-	++	+++	**6a**	++	+	-
3f	++	-	++	**6b**	+++	+++	++
3g	+	+	+++	**6c**	+	+	+
3h	++++	-	+++	**6d**	+++	+++	+++
4a	++	+	+++	**6e**	-	++	-
4b	+++	+++	++	**6f**	++	+++	+++
4c	+	+	++	**6g**	+	+	++
4d	++++	+	++	**6h**	+++	++++	+++

250 μg/mL= −, 200 μg/mL= +−, 150 μg/mL= −−, 100 μg/mL= −−++, 50 μg/mL= −−+++, Not active up to 250 μg/mL= - Standard drugs were Ciprofloxacin (MIC 5 μg/mL), Cloxacillin (MIC 10 μg/mL) and Gentamycin (MIC 5 μg/mL)

which have two methyl substituents, out of which one is at the 8th position of the benzopyrano ring are predicted to have significantly higher activity than the rest of the compounds.

EXPERIMENTAL

Melting points were taken in open capillaries and were uncorrected. IR spectra (ν_{max} in cm^{-1}) were recorded on Perkin Elmer FTIR and NMR (^{1}H and ^{13}C) was recorded on Bruker AMX 300 MHz using TMS as standard. Mass spectra were recorded on Shimadzu GC-MS. The homogeneity of the compounds was determined on the silica gel plates. The spots were developed in the iodine chamber. All the compounds gave satisfactory elemental analysis.

General Method for the synthesis of 1,1-Dicyano-2-[4′-hydroxy-2′*H*(1) benzopyran-2′-one-3′-yl] ethene/propene 2a-h

A mixture of 3-acetyl/formyl-4-hydroxy-2*H*(1)-benzopyran-2-one **1a-h** (0.01 mol) and malononitrile (0.01 mol) in alcohol and piperidine (0.8 ml) was stirred for 4 hr at room temperature. The reaction was monitored on TLC. It was then decomposed in crushed ice and neutralized with dilute HCl. The solid obtained was filtered, washed and recrystallized from methanol to give dicyano derivatives **2a-h.**

2a: mp: 198 ℃, yield: 67%.

IR (cm^{-1}): 3412(OH), 2245(CN), 1726(C=O), 1620, 1441, 1282, 1138, 784 cm^{-1}.

A.E.: Calcd (Found): C, 65.55(65.42); H, 2.52(2.30); N, 11.77(11.56)

2b: mp: 154℃, yield: 65%.

IR (cm^{-1}): 3362(OH), 2204(CN), 1735(C=O), 1618, 1424, 1278, 1147, 776 cm^{-1}.

A.E.: Calcd (Found): C, 66.66(66.54); H, 3.17(3.02); N, 11.11(11.13).

2c: mp: 178℃, yield: 72%.

IR (cm^{-1}): 3402(OH), 2205(CN), 1729(C=O), 1620, 1441, 1282, 1138, 784 cm^{-1}.

^{1}H NMR DMSO-d_6 (δ-ppm): 2.39 (s, 3H, CH3), 6.50 (s, 1H, CH=C $(CN)_2$), 6.94 (d, 1H, C_6-H, *J*=7.5 Hz), 7.05 (d, 1H, C_5-H, *J*=7.5Hz), 7.50 (s, 1H, C_8-H), 9.10 (s, 1H, OH, D_2O exchanged).

^{13}C NMR DMSO-d_6 (δ-ppm): 20.1 (CH3, at C7'), 100.0 (C-3'), 132.6 (C2), 118.2 (C10'), 114.9 (CN), 119.8 (C8'), 120.9 (C5'), 126.5 (C6'), 128.6 (C7'), 152.5 (C1 C(CN)2), 156.0 (C9'), 163.5 (C4'),165.0 (C2').

A.E.: Calcd (Found): C, 66.66(66.62); H, 3.17(3.19); N, 11.11(11.07).

2d: mp: 168 °C, yield: 71%.

IR (cm^{-1}): 3332(OH), 2212(CN), 1732(C=O), 1621, 1443, 1279, 1143, 782 cm^{-1}.

A.E.: Calcd (Found): C, 66.66(66.75); H, 3.17(3.13); N, 11.11(11.10).

2e: mp: 203 °C, yield: 75%.

IR (cm^{-1}): 3354(OH), 2210(CN), 1707(C=O), 1607, 1445, 1253, 1178, 824 cm^{-1}.

A.E.: Calcd (Found): C, 66.66(66.50); H, 3.17(3.00); N, 11.11(11.09).

2f: mp: 160 °C, yield: 59%.

IR (cm^{-1}): 3357(OH), 2199(CN), 1735(C=O), 1647, 1478, 1281, 1198, 770 cm^{-1}.

A.E.: Calcd (Found): C, 67.66(67.45); H, 3.75(3.90); N, 10.52(10.68).

2g: mp: 224°C, yield: 67%

IR (cm^{-1}): 3356(OH), 2202(CN), 1722(C=O), 1618, 1424, 1263, 818 cm^{-1}.

A.E.: Calcd (Found): C, 67.66(67.47); H, 3.75(3.88); N, 10.52(10.62).

2h: mp: > 300 °C, yield: 66%.

IR (cm^{-1}): 3356(OH), 2202(CN), 1722(>C=O), 1618, 1424, 1263, 1040, 818, 660.

^{1}H NMR DMSO-d_6 (δ-ppm): 2.36(s, 3H, CH_3), 2.64(s, 3H, CH_3), 7.35(t, 1H, C_6-H, *J*=8.5 Hz), 7.50 (d, 1H, C_7-H, *J*=8.5 Hz), 7.66(d, 1H, C_5-H, *J*=8.5 Hz), 9.70(s, 1H, OH, D_2O exchanged).

^{13}C NMR DMSO-d_6 (δ-ppm): 17.6(CH_3, at C_8'), 21.8 (C_3), 100(C_3'), 104.5(C_2), 112.7(C_{10}'), 116.6(CN), 118.8(C_8'), 125.5(C_5'), 128.5(C_6'), 133.3(C_7'), 135.0(C_2), 139.1(C_1, C(CN)$_2$), 153.4(C_9'), 161.8(C_4'), 165.0(C_2').

A.E.: Calcd (Found): C, 67.66(67.47); H, 3.75(3.65); N, 10.52(10.59).

General Method for the synthesis of 1,1-Di (1*H* tetrazol-5-yl) 2-[4'-hydroxy-2'*H* (1)-benzopyran-2'-one 3'-yl] ethene/propene 4a-h

Dicyano compounds **2** (0.01 mol), NH_4Cl (0.02 mol) and NaN_3 (0.02 mol) in DMF were refluxed at 130 °C for 19 hr. The cooled reaction mixture was then poured into crushed ice and acidified with HCl to pH 2. The suspension was kept at 4 °C for 12 h and was filtered. The residue was washed, dried and recrystallized from ethanol to gave ditetrazoles **4a-h.**

4a: mp: 225°C, yield: 47%.

IR (cm^{-1}): 3423(OH), 1727(C=O), 1625, 1447, 1277, 1156, 776 cm^{-1}.

A.E.: Calcd (Found): C, 48.15(48.02); H, 2.47(2.39); N, 34.57(34.49).

4b: mp: 213°C, yield: 64%.

IR (cm^{-1}): 3354(OH), 1733(C=O), 1633, 1445, 1245, 1146, 875 cm^{-1}.

A.E.: Calcd (Found): C, 49.70(49.60); H, 2.95(2.82); N, 33.14(33.06).

4c: mp: >300 °C, yield: 62%.

IR (cm^{-1}): 3410(OH), 1714(>C=O), 1619, 1448, 1259, 1050, 870, 789 cm^{-1}.

^{1}H NMR DMSO-d_6 (δ-ppm): 2.30 (s,3H,CH3), 6.40 (s,1H,CH=C(CN)2), 6.98 (d, 1H, C6/-H, *J*=7 Hz), 7.09 (d, 1H, C5/-H, *J*=7 Hz), 7.29 (s, 1H, C8/-H), 9.15 (s,2H,NH), 10.30 (s,1H,OH, D_2O exchangeable).

^{13}C NMR DMSOd_6 (δ-ppm): 20.1(CH_3, at C_7'), 100.0(C_3'), 102.0(C_2), 112.4(C_{10}'), 118.2(C_8'), 120.9(C_5'), 126.5(C_6'), 132.6(C_7'), 140.9(C_1), 152.5(C_9'), 163.5(C_4'), 165.0(C_2'), 156.0(N=C-N).

MS m/z (%): 338 (M+) (71), 299(56), 280(9), 264(13), 249(9), 229(6), 200(6) 178(6), 149(16), 137(84), 121(31), 105(47), 91(62), 77(100), 51(53) etc.

A.E.: Calcd (Found): C, 49.70(49.73); H, 2.95(2.78); N, 33.14(33.17).

4d: mp: 168 °C, yield: 54%.

IR (cm^{-1}): 3342(OH), 1724(C=O), 1643, 1456, 1288, 1134, 824.

A.E.: Calcd (Found): C, 49.70(49.68); H, 2.95(2.80); N, 33.14(33.12).

4e: mp: 230 °C, yield: 65%.

IR (cm^{-1}): 3442(OH), 1706(C=O), 1614, 1430, 1210, 1040, 825.

A.E.: Calcd (Found): C, 49.70(49.77); H, 2.95(3.02);

N, 33.14(33.19).

4f: mp: >300 ℃, yield: 57%.

IR (cm^{-1}): 3430(OH), 1710(C=O), 1609, 1442, 1207, 1064, 819.

A.E.: Calcd (Found): C, 51.11(51.07); H, 3.40(3.45); N, 31.81(31.92).

4g: mp: 231 ℃, yield: 49%.

IR (cm^{-1}): 3445(OH), 1720(C=O), 1620, 1450, 1370, 1050, 820.

A.E.: Calcd (Found): C, 51.11(51.15); H, 3.40(3.37); N, 31.81(31.90).

4h: mp: >300 ℃, yield: 55%.

IR (cm^{-1}): 3439(OH), 1726(C=O), 1619, 1428, 1376, 1200, 1058, 820 cm^{-1}.

^{1}H NMR DMSO-d_6 (δ-ppm): 2.30, 2.62(s, 6H, $2CH_3$), 7.28(t, 1H, C_6'-H, *J*= 8.0 Hz), 7.49(d, 1H, C_7'-H, *J*=8Hz), 7.65(d, 1H, C_5'-H, *J*=8Hz), 9.15(s, 2H, NH) and 10.15(s, 1H, OH, D_2O exchangeable).

^{13}C NMR DMSO-d_6 (δ-ppm): 17.6(CH_3, at C_8'), 20(CH_3, C_3), 100.5(C_3'), 102.2(C_2), 112.5(C_{10}'), 118.2(C_8'), 123.3(C_5'), 125.6(C_6'), 133.6(C_7'), 150.8(C_1), 151.8(C_9'), 161.8(C_4'), 165.9(C_2'), 170.0(N=C-N).

MS m/z(%): 352 (M^+)(71),322(56), 299(9), 283(13), 264(9), 245(6), 229(6), 214(6), 204(16), 184(84), 176(31), 167(47), 150(62), 145(22), 137(56), 121(31), 108(56), 95(31), 91(40), 77(100), 69(43), 56(56), 41(53).

A.E.: Calcd (Found): C, 51.11(51.18); H, 3.40(3.48); N, 31.81(31.85).

General Method for the synthesis of Ethyl-2-cyano-3-[4′-hydroxy-2′*H*(1)-benzopyran-2′-one-3′-yl] propenoate/ butenoate 3a-h

A mixture of 3-acetyl/formyl-4-hydroxy-2*H*(1)-benzopyran-2-one **1a-h** (0.01 mol) and ethyl cyanoacetate (0.01 mol) in alcohol in presence of piperidine (1 ml) was refluxed for 12 hr. on water-bath. The reaction was monitored on TLC. After the completion of reaction it was decomposed in crushed ice, neutralized with dilute HCl. The solid obtained was filtered, washed and recrystallised from ethanol to gave cyano esters **3a-h**.

3a: mp: 164℃, yield: 65%.

IR (cm^{-1}): 3415(OH), 2243(CN), 1725(C=O), 1621, 1443, 1283, 1134, 785.

A.E.: Calcd (Found): C, 63.16(63.10); H, 3.86(3.79); N, 4.91(4.89).

3b: mp: 159 ℃, yield: 62%

IR (cm^{-1}): 3342(OH), 2214(CN), 1737(C=O), 1614, 1426, 1274, 1143, 768.

A.E.: Calcd (Found): C, 64.21(64.11); H, 4.35(4.14); N, 4.68(4.72).

3c: mp: 280 ℃, yield: 72%.

IR (cm^{-1}): 3401(OH), 2204(CN), 1731(>C=O), 1670(>C=O), 1600, 1442, 1393, 1281, 1186, 1138, 790.

^{1}H NMR DMSO-d_6 (δ-ppm): 1.30 (t, 3H, CH3), 2.40(s, 3H, CH3), 4.34(q, 2H, CH2), 6.45(s, 1H, C2-H), 7.20(d, 1H, C6/H, *J*=7.5 Hz), 7.30(d, 1H, C5/-H, *J*=7.5 Hz), 7.50 (s,1H, C8/H), 10.90 (s,1H, OH, D_2O exchanged).

^{13}C NMR DMSO-d_6 (δ-ppm): 15.0 ($\underline{C}H_3CH_2$), 20.0 (CH_3), 56.0 (OCH_2), 100.6 (C_3'), 132.6 (C_3), 118.2 (C_{10}'), 114.9 (CN), 119.8 (C_8'), 120.9 (C_5'), 126.5 (C_6'), 128.6 (C_7'), 154.3 (C_9'), 151.2 (C_2), 161.8 (C_4'), 163.5 (C_2'), 166.9 (C_1, $\underline{C}OO$).

A.E.: Calcd (Found): C, 64.21(64.18); H, 4.35(4.26); N, 4.68(4.59).

3d: mp: 163 ℃, yield: 68%.

IR (cm^{-1}): 3341(OH), 2219(CN), 1723(C=O), 1642, 1446, 1283, 1149, 781 cm^{-1}.

A.E.: Calcd (Found): C, 64.21(64.24); H, 4.35(4.31); N, 4.68(4.63).

3e: mp: 140 ℃, yield: 75%.

IR (cm^{-1}): 3423(OH), 2199(CN), 1705(C=O), 1619, 1461, 1258, 789.

A.E.: Calcd (Found): C, 64.21(64.48); H, 4.35(4.10); N, 4.68(4.50).

3f: mp: 118℃, yield: 59%.

IR (cm^{-1}): 3400(OH), 2210(CN), 1710(C=O), 1644, 1461, 1254, 1179, 759.

A.E.: Calcd (Found): C, 65.17(65.30); H, 4.79(4.85); N, 4.47(4.55).

3g: mp: 160 ℃, yield: 67%

IR (cm^{-1}): 3414(OH), 2211(CN), 1713(C=O), 1645, 1461, 1254, 827.

A.E.: Calcd (Found): C, 65.17(65.20); H, 4.79(4.75); N, 4.47(4.51).

3h: mp: 180 ℃, yield: 66%.

IR (cm^{-1}): 3337(OH), 2202(CN), 1723(C=O), 1615, 1452, 1290, 1065, 819, 785.

^{1}H NMR DMSO-d_6 (δ-ppm): 1.38 (t, 3H, $CH_2\underline{C}H_3$), 2.10(s, 3H, CH_3), 2.50(s, 3H, CH_3), 4.39(q, 2H, CH_2), 7.17(t, 1H, C_6-H, J= 8.0 Hz), 7.39(d, 1H, C_7-H, J=8.0 Hz), 7.58(d, 1H, C_5-H, J=8.0 Hz) 10.80 (s, 1H, OH, D_2O exchanged).

^{13}C NMR DMSO-d_6 (δ-ppm): 17.7($\underline{C}H_3CH_2$), 18.2 (C_4, CH_3), 20.7 (CH_3 at C_8), 52(OCH_2), 99.9(C_3), 102.8(C_3), 113.1(C_{10}), 115.4(CN), 118.5(C_8), 123.3 (C_5), 125.1(C_6), 133.6(C_7, C-CH_3), 151.8(C_9), 155.3 (C_2), 161.8(C_4), 165.8(C_2), 170.8(C_4,COO).

Ms m/z (%): 313(M^-), 266, 240, 236, 214, 204, 190, 184, 175, 167, 161, 150, 137, 121, 108, 95, 77, 56, 42.

A.E.: Calcd (Found): C, 65.17(65.23); H, 4.79(4.70); N, 4.47(4.41).

General Method for the synthesis of 3-Cyano-2*H*,5*H*-pyrano[3,2-*c*] benzopyran-2,5-dione 5a-d and 4-Methyl-3-cyano-2*H*,5*H*-pyrano[3,2-*c*] benzopyran-2,5-dione 5e-h.

Cyano esters **3a-h** (0.01 mol) was heated with PPA (30 ml) at 130-135°C for 15 hrs. The reaction was monitored on TLC. After the completion of reaction, the reaction mixture was cooled and decomposed in crushed ice. The solid obtain was filtered, washed with water and recrystallized from ethanol to gave 3-cyano pyrano benzopyrans **5a-h**.

5a: mp: 222°C, yield: 56%.

IR (cm^{-1}): 2233(CN), 1734(C=O), 1628, 1436, 1276, 1142, 781.

A.E.: Calcd (Found): C, 65.27(65.09); H, 2.09(2.00); N, 5.86(5.82).

5b: mp: 165 °C, yield: 61%

IR (cm^{-1}): 2208(CN), 1723(C=O), 1623, 1446, 1264, 1137, 771.

A.E.: Calcd (Found): C, 66.40(66.22); H, 2.77(2.65); N, 5.53(5.50).

5c: mp: >300 °C, yield: 69%.

IR (cm^{-1}): 2203(CN), 1716(>C=O), 1647, 1618, 1444, 1258, 1147, 1080, 788.

^{1}H NMR DMSO-d_6 (δ-ppm): 2.25(s, 3H, CH3), 6.75(s, 1H, C4-H), 6.97(d, 1H, C9-H, J=7.5 Hz), 7.10(d, 1H, C10-H, J=7.5Hz), 7.31(s, 1H, C7-H).

^{13}C NMR DMSO-d_6 (δ-ppm): 20.1(CH_3), 100.0 (C_{4a}), 115.9(CN), 119.9 (C_7), 121.0(C_9), 122.4(C_{10}), 128.0(C_{10a}), 130.0(C_4), 134.0(C_8), 150.9(C_3), 152.8 (C_{6a} and C_{11}), 161.8(C_5), 162.4(C_2).

A.E.: Calcd (Found): C, 66.40(66.35); H, 2.77(2.58); N, 5.53(5.47).

5d: mp: 174 °C, yield: 64%.

IR (cm^{-1}): 2206(CN), 1732(C=O), 1644, 1452, 1279, 1152, 784.

A.E.: Calcd (Found): C, 66.40(66.47); H, 2.77(2.73); N, 5.53(5.52).

5e: mp: 248°C, yield: 65%.

IR (cm^{-1}): 2200(CN), 1725(C=O), 1605, 1481, 1268, 770.

A.E.: Calcd (Found): C, 66.40(66.25); H, 2.77(2.80); N, 5.53(5.45).

5f: mp: 145 °C, yield: 59%.

IR (cm^{-1}): 2202(CN), 1723(C=O), 1615, 1452, 1290, 785.

A.E.: Calcd (Found): C, 67.42(67.55); H, 3.39(3.41); N, 5.24(5.58).

5g: mp: 205 °C, yield: 62%.

IR (cm^{-1}): 2201(CN), 1720(C=O), 1620, 1555, 821.

A.E.: Calcd (Found): C, 67.42(67.48); H, 3.39(3.31); N, 5.24(5.35).

5h: mp: 210 °C, yield: 52%.

IR (cm^{-1}): 2196(CN), 1733(C=O), 1647, 1547, 1263, 1188, 1088, 1027, 772.

^{1}H NMR DMSO-d_6 (δ-ppm): 2.35(s, 3H, CH_3, C_8), 2.71(s, 3H, CH_3, C_4), 7.30(d, 1H, C_8-H, J=8.0 Hz), 7.47(t, 1H, C_9-H, J=8.0 Hz), 7.85(d, 1H, C_{10}-H, J=8.0 Hz).

^{13}C NMR DMSO-d_6 (δ-ppm): 15.2(CH_3, C_4), 19.9(CH_3, C_7), 101.3(C_4), 107.5(C_3), 113.1(C_{10a}), 115.4 (C_{4a}), 118.4(CN), 120.8(C_7), 123.3(C_{10}), 125.1(C_9), 133.6(C_8), 151.8(C_{6a} and C_{11}), 165.8(C_2 and C_5).

MS m/z (%): 267(M^-), 241, 190, 177, 175, 157, 150, 144, 136, 121, 115, 108, 91, 84, 77, 63, 51, 44.

A.E.: Calcd (Found): C, 67.42(67.51); H, 3.39(3.33); N, 5.24(5.28).

General Method for the synthesis of 3-(1′*H*-tetrazol-5′-yl)-2*H*, 5*H*-pyrano[3,2-*c*]benzopyran-2,5-dione 6a-d 4-Methyl-3-(1′*H*-tetrazol-5′-yl)-2*H*,5*H*-pyrano[3,2-*c*]benzopyran-2,5-dione 6e-h.

A mixture of 3-cyano pyranobenzopyrans **5** (0.01 mol), NH_4Cl (0.01 mol) and NaN_3 (0.01 mol) in DMF were refluxed for 21 hr. The reaction mixture was cooled, poured into crushed ice and acidified

with HCl to pH 2. The suspension was kept at 4℃ for 12 h and was filtered; the residue was washed dried and recrystallized from ethanol to gave 3-tetrazole pyrano benzopyrans **6a-h**.

6a: mp: 217 ℃, yield: 59%.

IR (cm^{-1}): 3341, 3215(NH), 1737(C=O), 1653, 1632, 1445, 1269, 1143, 786.

A.E.: Calcd (Found): C, 55.32(55.34); H, 2.13(2.08); N, 19.86(19.80).

6b: mp: 254℃, yield: 65%

IR (cm^{-1}): 3412, 3398(NH), 1726(C=O), 1673, 1618, 1436, 1266, 1141, 770.

A.E.: Calcd (Found): C, 56.76(56.60); H, 2.70(2.67); N, 18.92(18.79).

6c: mp: 270℃, yield: 69%.

IR (cm^{-1}): 3443, 3210(NH), 1720(>C=O), 1640, 1620, 1516, 1394, 1267, 1174, 1071, 871, 791.

^{1}H NMR DMSO-d_6 (δ-ppm): 2.30 (s, 3H, CH_3), 6.70 (s, 1H, C_4-H), 7.20 (d, 1H, C_9-H, *J*=8.5 Hz), 7.31 (d, 1H, C_{10}-H, *J*=8.5Hz), 7.49 (s, 1H, C_7-H), 9.15 (s, 1H, NH, D_2O exchanged).

^{13}C NMR DMSO-d_6 (δ-ppm): 20.1 (CH_3), 101.5 (C_{4a}), 119.5 (C_7), 121.0 (C_9), 122.0 (C_{10}), 127.5 (C_{10a}), 129.5 (C_4), 132.6 (C_8), 152.2 (C_{6a} and C_{11}), 155.3 (N-C=N), 159.1 (C_3), 162.1(C_5), 164.5 (C_2).

MS m/z (%): 296(M^-), 277(25), 227(47), 214(6), 190(9), 175(28), 161(25), 150(100), 143(25), 137(60), 121(75), 105(56), 91(49), 77(97), 44(49).

A.E.: Calcd (Found): C, 56.76(56.80); H, 2.70(2.72); N, 18.92(18.86).

6d: mp: 264℃, yield: 66%.

IR (cm^{-1}): 3345, 3214(NH), 1723(C=O), 1667, 1643, 1451, 1273, 1151, 783.

A.E.: Calcd (Found): C, 56.76(56.71); H, 2.70(2.61); N, 18.92(18.82).

6e: mp: >300 ℃, yield: 68%.

IR (cm^{-1}): 3440, 3378(NH), 1726(C=O), 1662, 1619, 1498, 1298, 819.

A.E.: Calcd (Found): C, 56.75(56.68); H, 3.37(3.31); N, 18.92(18.95).

6f: mp: 207℃, yield: 57%.

IR (cm^{-1}): 3440, 3380(NH), 1727(C=O), 1650, 1620, 1499, 1201, 820.

A.E.: Calcd (Found): C, 58.06(57.98); H, 3.22(3.30); N, 18.06(18.09).

6g: mp: 233 ℃, yield: 72%.

IR (cm^{-1}): 3440, 3380, 1727(C=O), 1650, 1620, 1499, 1201, 819.

A.E.: Calcd (Found): C, 58.06(58.10); H, 3.22(3.15); N, 18.06(18.00).

6h: mp: 267 ℃, yield: 60%.

IR (cm^{-1}): 3439, 3500(NH), 1726(C=O), 1663 (>C=N), 1619, 1428, 1376, 1298, 1201, 1058, 820.

^{1}H NMR DMSO-d_6 (δ-ppm): 2.15(s, 3H, CH_3, C_8), 2.61(s, 3H, CH_3, C_4), 7.25(t, 1H, C_9-H, *J*=7.0 Hz), 7.44(d, 1H, C_8-H, *J*=7.0 Hz), 7.58(d, 1H, C_{10}-H, *J*=7.0 Hz), 8.88(s, 1H, NH, D_2O exchanged).

^{13}C NMR DMSO-d_6 (δ-ppm): 17.5(CH_3, C4), 21.2 (CH_3, C_7), 100.8(C_4), 110.2(C_3), 112.0(C_{10a}), 117.1(C_{4a}), 125.0(C_7), 126.1(C_{10}), 125(C_9), 135(C_8), 153.1(C_{6a} and C_{11}), 158(N-C=N), 167.1(C_2 and C_5).

MS m/z (%): 310(M^+) (56), 241(25), 236(47), 229(6), 215(9), 204(16), 184(49), 178(28), 150(100), 145(25), 137(60), 121(75), 108(56), 95(49), 77(97), 42(49), 41(40).

A.E.: Calcd (Found): C, 58.06(58.12); H, 3.22(3.17); N, 18.06(17.98).

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