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강한 항균제인 Pyranobenzopyran 유도체의 합성

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Synthesis of Pyranobenzopyran Derivatives as a Potent Antibacterial Agent

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요 약. 피페리딘 존재하에서 디에틸 말론산염과 3-Formyl-4-hydroxy-2*H*(1)-benzopyran-2-one(**1a-d**)를 사용하여 ethyl-2*H*,5*H*-2,5-dioxopyrano[3,2-*c*] benzopyran-3-oate(**2a-d**)을 합성하였다. 아세토 아세트산 에틸을 사용한 **2a-d**의 마이클 첨가반응을 사용하여 ethyl 2,4-dihydroxy-5*H*, 12*H*- 5,12-dioxo [2] benzo pyrano [4,3-*c*] [1] benzopyran-1-oate(**3a-d**)을 합성하였다. 3a-d와 베크만 축합반응물과 아세토 아세트산 에틸은 2-acetyl-1,6-dihydroxy-3*H*,7*H*,14*H*-3,7,14-trioxo-pyrano[2',3',5,6][2] benzo pyrano[3,2-*c*] benzopyran(**4a-d**)이 되었다. 메톡사이드나트륨의 존재하에 끓는 메탄올에서 마이클 첨가에 뒤이어 아세틸 아세톤의 고리화 반응으로 ethyl-2*H*,5*H*-2,5-dioxopyrano[3,2-*c*] benzopyran-3-oate(**2a-d**)을 사용하여 1-acetyl-2-methyl-4*H*,5*H*,12*H*,4,5,12-trioxo-dipyrano[3,4-d;3',2'-*c*] benzopyran(**5a-d.**)을 합성하였다.

주제어: 3-Formyl-4-hydroxy-2*H*(1)-benzopyran-2-one, Pyranobenzopyran, 마이클 첨가, 베크만 축합, 항균활성도

ABSTRACT. 3-Formyl-4-hydroxy-2*H*(1)-benzopyran-2-one **1a-d** on reaction with diethyl malonate in the presence of piperidine give ethyl-2*H*,5*H*-2,5-dioxopyrano[3,2-*c*] benzopyran-3-oate. **2a-d.** Michael addition of **2a-d** with ethyl aceto acetate gives ethyl 2,4-dihydroxy-5*H*, 12*H*- 5,12-dioxo [2] benzo pyrano [4,3-*c*] [1] benzopyran- 1-oate (3a-d). **3a-d** on Pechmann condensation with ethyl aceto acetate gives 2-acetyl-1,6-dihydroxy-3*H*,7*H*,14*H*-3,7,14-trioxo-pyrano[2',3',5,6] [2] benzo pyrano [3,2-*c*] benzopyran **4a-d.** Michael addition followed by cyclisation of acetyl acetone with ethyl-2*H*,5*H*-2,5-dioxo-pyrano[3,2-*c*] benzopyran-3-oate **2 a-d** in the presence of sodium methoxide in boiling methanol afforded1-acetyl-2-methyl-4*H*,5*H*,12*H*,4,5,12-trioxo-dipyrano[3,4-d;3',2'-*c*] benzopyran. **5a-d.**

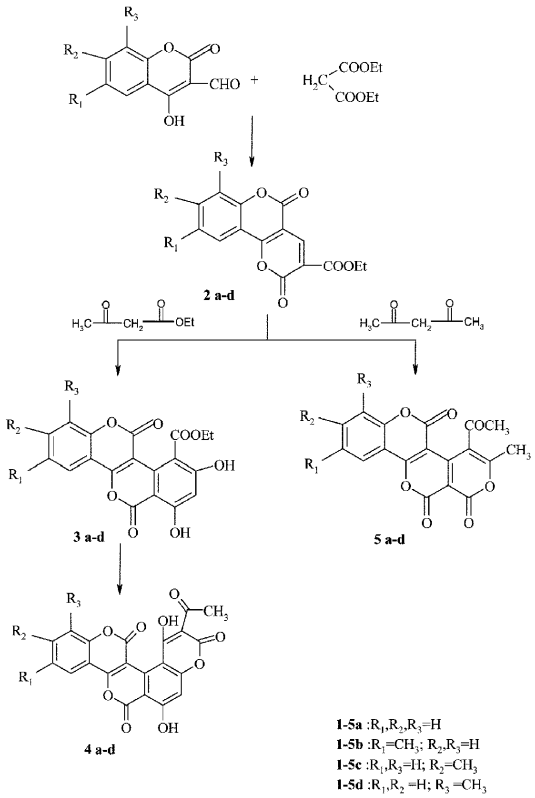
Keywords: 3-Formyl-4-hydroxy-2*H*(1)-benzopyran-2-one, Pyranobenzopyran, Michael Addition, Pechmann Condensation, Antimicrobial Activity

INTRODUCTION

Pyranobenzopyrans and its derivatives are reported to possess various biological activities[1-10] such as antibacterial, antifungal, CNS depressant, antiviral, ulcer inhibitor etc. Suksdorfin and DCK which contain pyranobenzopyran moiety are known to be potent anti -HIV agents.[3] Similarly suksdorfin inhibited HIV-1 replication in H_9 lymphocytes with an in vitro EC_{50} value of 1.3 μm and a therapeutic index of value of >40, while DCK showed extremely potent inhibitory activity against HIV-1.Pyranobenzopyrans are also gaining importance in laser dyes.[11] The biological importance of pyranobenzopyran impressed us to synthesize new heterocyclic compounds which contain pyranobenzopyran moieties. All the synthesized compounds were screened for their antibacterial activity.

RESULTS AND DISCUSSION

In order to synthesize ethyl-2*H*,5*H*-2,5-dioxopyrano[3,2-*c*] benzopyran-3-oate. **2a-d**, the Knoevengeal condensation of 3-formyl-4-hydroxy-2*H*(1)-benzopyran-2-one[12] **1a-d** with diethyl malonate was carried out in the presence of piperidine to afford the above compound in quantitative yield. With an interest to carry out Michael addition, ethyl-2*H*,5*H*-2,5-dioxopyrano[3,2-*c*] benzopyran-3-oate **2a-d** and ethyl aceto acetate were heated in presence of sodium methoxide to give ethyl 2,4-dihydroxy-5*H*, 12*H*- 5,12-dioxo [2] benzo pyrano [4,3-*c*] [1] benzopyran- 1-oate **3a-d**. Solution of **3a-d** in NaOH gives coloration with $FeCl_3$. **3a-d** on Pechmann condensation with ethyl aceto acetate gives 2-acetyl-1,6-dihydroxy-3*H*,7*H*,14*H*-3,7,14-trioxo-pyrano[2',3',5,6] [2] benzo pyrano [3,2-*c*] benzopyran **4a-d**. Michael addition followed by cyclisation of acetyl acetone with ethyl-2*H*,5*H*-2,5-dioxo-pyrano[3,2-*c*] benzopyran-3-oate **2 a-d** in the presence of sodium methoxide in boiling absolute methanol afforded 1-acetyl-2-methyl-4*H*,5*H*,12*H*,4,5,12-trioxo-dipyrano [3,4-d;3',2'-*c*] benzopyran **5a-d**. The structures of the compounds 2a-d to 5a-d were confirmed on the basis of spectral and analytical data. These synthetic reactions are summarized in *Scheme* 1.



Scheme 1.

All the above synthesized compounds were screened *in vitro* for their antimicrobial activity against vari-

Table 1. Characterization data of compounds **2a-d**, **3a-d**, **4a-d**, **5a-d**

Com.	Mol. Formula	R_1 R_2 R_3	M. P. °C	Yield (%)	Elemental analysis [Found (Cal.) (%)]	
					C	H
2a	$C_{15}H_{10}O_6$	H H H	214	67	62.42(62.94)	3.30(3.49)
2b	$C_{16}H_{12}O_6$	CH_3 H H	223	65	63.54(64.00)	3.82(4.00)
2c	$C_{16}H_{12}O_6$	H CH_3 H	219	71	63.62(64.00)	3.89(4.00)
2d	$C_{16}H_{12}O_6$	H H CH_3	212	70	63.75(64.00)	3.93(4.00)
3a	$C_{19}H_{12}O_8$	H H H	230	64	61.87(61.96)	3.23(3.26)
3b	$C_{20}H_{14}O_8$	CH_3 H H	257	69	62.69(62.83)	3.59(3.67)
3c	$C_{20}H_{14}O_8$	H CH_3 H	254	63	62.81(62.83)	3.64(3.67)
3d	$C_{20}H_{14}O_8$	H H CH_3	249	67	62.78(62.83)	3.61(3.67)
4a	$C_{21}H_{10}O_9$	H H H	256	63	61.96(62.07)	2.38(2.46)
4b	$C_{22}H_{12}O_9$	CH_3 H H	261	66	62.79(62.86)	2.83(2.86)
4c	$C_{22}H_{12}O_9$	H CH_3 H	258	66	62.82(62.86)	2.79(2.86)
4d	$C_{22}H_{12}O_9$	H H CH_3	253	63	62.80(62.86)	2.82(2.86)
5a	$C_{18}H_{10}O_7$	H H H	236	49	62.99(63.91)	2.91(2.96)
5b	$C_{19}H_{12}O_7$	CH_3 H H	243	52	64.17(64.77)	3.35(3.41)
5c	$C_{19}H_{12}O_7$	H CH_3 H	247	48	64.52(64.77)	3.25(3.41)
5d	$C_{19}H_{12}O_7$	H H CH_3	240	50	64.35(64.77)	3.29(3.41)

Table 2. Antibacterial activity of compounds **2a-d, 3a-d, 4a-d, 5 a-d**

Compound	Antibacterial μg/mL			Compound	Antibacterial μg/mL		
	S. aureus	*S. typhi*	*E. coli*		*S. aureus*	*S. typhi*	*E. coli*
2a	-	-	-	4a	110	130	110
2b	-	-	-	4b	110	125	120
2c	-	-	-	4c	115	125	110
2d	-	-	-	4d	115	120	105
3a	140	140	150	5a	125	135	125
3b	135	150	145	5b	125	135	130
3c	150	140	140	5c	130	135	130
3d	135	150	150	5d	120	120	140

=Not active up to 150 μg/mL.

Std: Ciprofloxacin (5 μg/mL); Cloxacillin (10 μg/mL); Gentamycin (5 μg/mL).

ety of bacterial strains. Gram negative strains of bacteria used were *S. typhi* and *E.coli* while gram positive bacterial strain used was *S.aureus*. The minimum inhibition concentration (MIC) was determined using Tube Dilution technique according to standard procedure[13] (*Table* 2). The standard drugs used for comparison were ciprofloxacin, cloxacillin and gentamycin. By visualizing the antimicrobial data it could be observed that many of the compounds possess significant antibacterial activity.

CONCLUSION

The antibacterial activity of the compounds 2-5(a-d) was compared and it was found that amongst them compound 3-5(a-d) showed significant activity against *S.aureus, S. typhi* and *E. coli*. due to fused pyranonebenzopyranone ring system. Compound 3b and 3d with methyl substitution at C-8 and C-10 showed significant antibacterial activities against *S. aureus*. Compounds 4a-4d showed comparable antibacterial activity against *S. aureus* and *E. coli*. Compound 4d having methyl group at C-12 position showed very significant activity against *E. coli*. The compound 5d having methyl group at C-10 position showed higher activity against *S. aureus* and *S. typhi*.

EXPERIMENTAL

Melting points were taken in open capillaries and are uncorrected. The IR spectra were recorded on a Perkin-Elmer 257 spectrometer using KBr discs. ^{1}H NMR and ^{13}C NMR spectras in DMSO-d_6 were recorded on VXR-300 MHz using TMS as internal standard and mass spectra were recorded on Shimadzu GC-MS. The homogeneity of the compounds was described by TLC on silica gel plates. The spots are developed in iodine chamber.

Ethyl-2*H*, 5*H*-2, 5-dioxo-pyrano [3, 2-*c*] benzopyran-3-oate 2a-d:

A mixture of 3-formyl-4-hydroxy-2*H*(1)-benzopyran-2-one **1a-d** (0.01 mol), diethyl malonate (0.01 mole) and piperidine (0.5 mL) was heated on water bath for 2 hr. The mixture was left at room temperature for 3 hr. and then it was poured into ice-cold water and acidified to get solid. The solid was filtered, washed with sodium bicarbonate and finally with water, dried and recrystallized from ethanol to get ethyl-2*H*,5*H*-2,5-dioxopyrano[3,2-*c*] benzopyran-3-oate. **2a-d.**

Ethyl 2, 4-dihydroxy-5*H*, 12*H*- 5, 12-dioxo [2] benzo pyrano [4, 3-*c*] [1] benzopyran- 1-oate 3a-d:

A mixture of ethyl-2*H*, 5*H*-2, 5-dioxopyrano [3, 2-*c*] benzopyran-3-oate **2a-d** (0.01 mol), ethyl aceto acetate (0.01 mol) and sodium methoxide (0.01 mol) was heated at 170 °C for 8 hr. The reaction mixture was cooled, triturated with cold hydrochloric acid. The solid obtained was filtered, washed with water, dried and recrystallized from methanol to get ethyl 2,4-dihydroxy-5*H*,12*H*-5,12-dioxo[2] benzo pyrano [4,3-*c*] [1] benzopyran-1-oate **3a-d.**

2-Acetyl-1,6-dihydroxy-3*H*, 7*H*, 14*H*, 3, 7,14-trioxo-pyrano [2′,3′, 5,6] [2] benzo pyrano [3,2-*c*] benzopyran. 4 a-d.

Ethyl 2, 4-dihydroxy-5*H*, 12*H*, 5, 12-dioxo [2] benzo

pyrano [4,3-*c*] [1] benzopyran 1-oate **3 a-d** (0.01 mol) and ethyl aceto acetate (0.01 mol) were taken in methanol (10 mL), to it piperidine (1.5 mL) was added and was refluxed for 8hr. The methanol was recovered in rotaevaporator. The reaction mixture was then decomposed into crushed ice and neutralized with dilute HCl to afforded solid product. The solid obtained was filtered, washed with water, dried and recrystallized from methanol to get 2-acetyl-1,6-dihydroxy-3*H*,7*H*,14*H*,3,7,14-trioxo-pyrano[2′, 3′,5,6] [2] benzo pyrano [3,2-*c*] benzopyran **4 a-d.**

1-Acetyl-2-methyl-4*H*,5*H*,12*H*,4,5,12-trioxo-dipyrano [3,4-d;3′,2′-*c*]benzopyran 5a-d.

To a solution of sodium methoxide (0.01 mol) in absolute methanol (10 mL), acetyl acetone (0.01 mol) was added and the solution was refluxed for 20 min. then ethyl-2*H*,5*H*-2,5-dioxopyrano[3,2-*c*] benzopyran-3-oate **2a-d** (0.01 mol) was added and the reaction mixture was refluxed for 5 hr. The methanol was recovered in rota evaporator. The reaction mixture was then decomposed into crushed ice and neutralized with dilute HCl to get the solid product. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol to get 1-acetyl-2-methyl-4*H*,5*H*,12*H*,4,5,12-trioxo-dipyrano[3,4-d;3′,2′-*c*] benzopyran. **5 a-d.**

2b. IR (KBr): 1719, 1614, 1527, 1429, 1375, 1297, 1209, 1043, 814, etc cm^{-1}.

^{1}H NMR DMSO-d_6 (δ-ppm): 1.25 (t, 3H, CH3), 2.19 (s, 3H, CH3), 3.9 (q, 2H, -OCH2), 7.1 (s, 1H, C4-H), 7.3 (s, 1H, C10-H), 7.59 (d, 1H, C8-H, J = 7.0 Hz), 7.8 (d, 1H, C7-H, J = 7.0 Hz).

Mass m/z (relative intensity, %): 300(26) (M^-), 272(10), 244(13), 228(7), 200(9), 175(23), 172(17), 149(23), 144(19), 116(37), 91(100), 77(26) etc.

3b. IR (KBr): 3420, 2924, 2363, 1731, 1659, 1620, 1447, 1384, 1315, 1230, 1175, 1057, 906, 872, 815, 789, etc. cm^{-1}.

^{1}H NMR DMSO-d_6 (δ-ppm): 1.22 (t, 3H, CH3), 2.1 (s, 3H, CH3), 4.25 (q, 2H, -OCH2), 5.6 (s, 1H, -OH, D_2O exchangeable), 6.7 (s, 1H, C_3-H), 7.05 (s, 1H, C_7-H), 7.8 (d, 1H, C_9-H, J = 7.5 Hz), 8.05 (d, 1H, C_{10}-H, J = 7.5 Hz), 9.5 (s, 1H, -OH, D_2O exchangeable).

Mass m/z (relative intensity, %): 382(24) (M^-) 354(10), 342(9),336(6), 326(14), 308(12), 298(7), 280(9), 252(8), 224(11), 209(23), 158(19), 150(28), 134(6), 106(34), 91(66), 84(47), 83(39), 77(100), 68(51), 57(55) etc.

4b. IR (KBr): 3433, 2944, 1723, 1621, 1577, 1494, 1426,1374, 1296, 1209, 1118, 1045, 817, etc. cm^{-1}

^{1}H NMR $CDCl_3$ (δ-ppm): 2.0 (s, 3H, CH3), 2.1 (s, 3H, CH_3), 5.8 (s, 1H, -OH, D_2O exchangeable), 6.8 (s, 1H, C_3-H), 7.1 (s, 1H, C_9-H), 7.4 (d, 1H, C_{11}-H, J = 7.0 Hz), 8.1 (d, 1H, C_{12}-H, J = 7.0 Hz), 9.8 (s, 1H, -OH, D_2O exchangeable).

Mass m/z (relative intensity, %): 420 (31) (M^+) 404(14), 378(10), 350(17), 336(13), 322(10), 308(6), 294(18), 280(14), 252(12),237(20), 200(15), 174(35), 134(64), 106(100), 91(62), 77(79) etc.

5b. IR (KBr): 2925, 2357, 1731, 1658, 1620, 1448, 1385, 1315, 1228, 1176, 1057, 788 etc. cm^{-1}.

^{1}H NMR DMSO-d_6 (δ-ppm): 2.05 (s, 3H, CH_3), 2.15 (s, 6H, 2 CH_3), 6.9 (s, 1H, C_7-H), 7.39 (d, 1H, C_9-H, J = 7.5 Hz), 7.9 (d, 1H, C_{10}-H, J = 7.5 Hz).

Mass m/z (relative intensity, %): 352(19) (M+), 338(8), 310(10), 299(5), 282(6), 254(6), 249(10), 226(13), 211(23), 196(27), 178(20), 149(30), 137(60), 121(23), 105(41), 91(100), 77(67), 51(53), etc.

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