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Synthesis and antibacterial activity of new oxadiazolo[1,3,5]-triazine, 1,2,4 triazolo and thiadiazolo 1,3,4 oxadiazole derivatives

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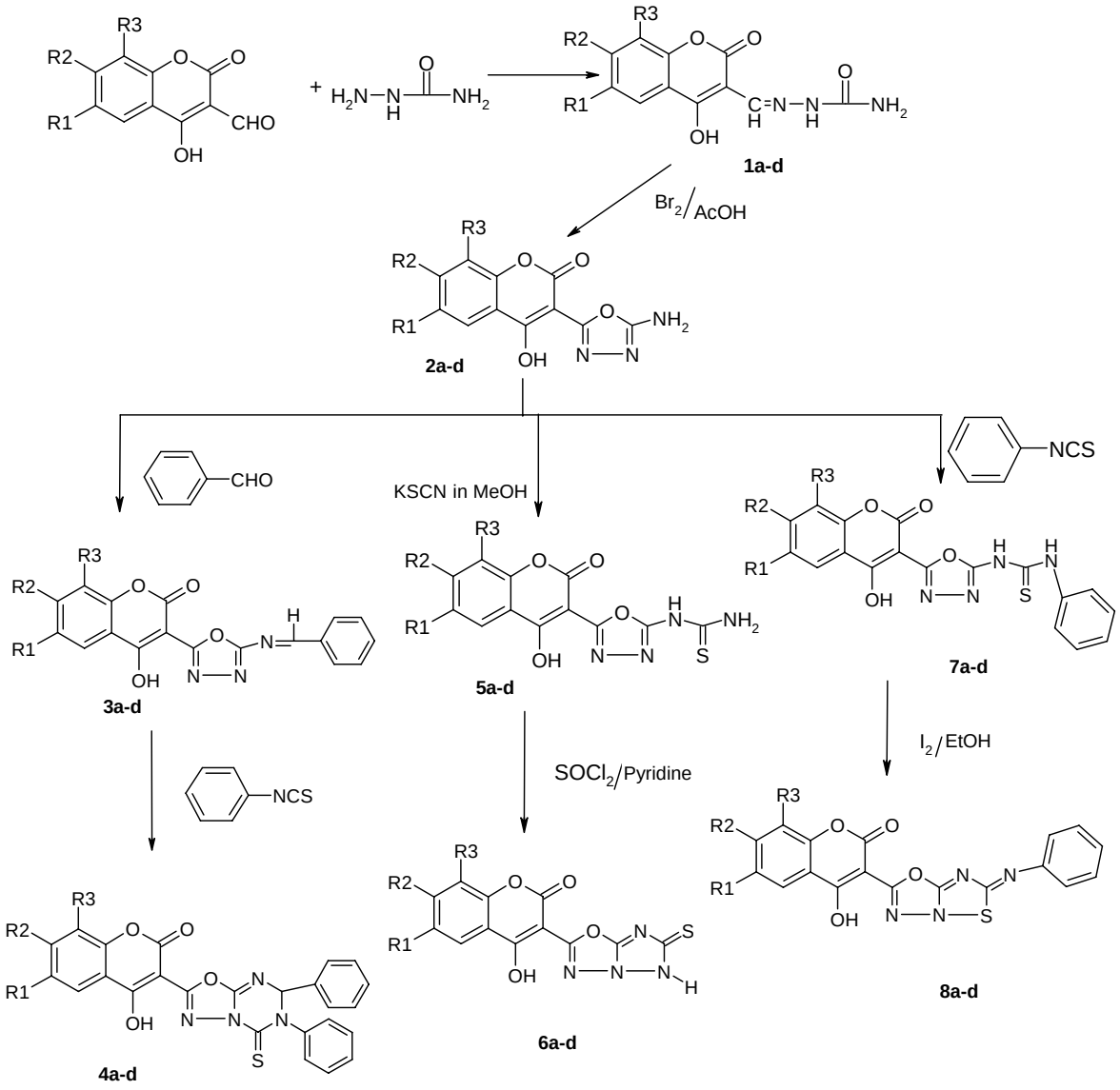
3-Formyl-4-hydroxycoumarin has been treated with semicarbazide to give 4-hydroxy-2-oxo-2*H*[1]-benzopyran-3-aldehyde semicarbazone **1a-d**, which on oxidative cyclization with bromine in glacial acetic acid in the presence of anhydrous sodium acetate gives 3-(5-amino-1,3,4-oxadiazol-2-yl)-4-hydroxy-2*H*[1]-benzopyran-2-one **2a-d**. 3-(5-amino-1,3,4-oxadiazol-2-yl)-4-hydroxy-2*H*[1]-benzopyran-2-one on reaction with benzaldehyde gives 4-hydroxy-3-(5-benzylidine imino 1,3,4-oxadiazol-2-yl)-2*H*[1]-benzopyran-2-one **3a-d**. **3a-d** on (4+2) cycloaddition with phenyl isothiocynate gives 3-(6,7-diphenyl-5-thioxo-6,7-dihydro-5*H*-[1,3,4]oxadiazolo[3,2-*a*][1,3,5]triazin-2-yl)-4-hydroxy-2*H*[1]-benzopyran-2-one **4a-d**. **2a-d** undergoes regioselective condensation with KSCN in methanol to give N-[5-(4-hydroxy-2-oxo-2*H*[1]-benzopyran-3-yl)-1,3,4,-oxadiazol-2-yl]thiourea **5a-d** whereas with phenyl isothiocynate it gives N-[5-(-4-hydroxy-2-oxo-2*H*[1]benzopyran-3-yl)-1,3,4-oxadiazole-2-yl]-N′-phenylthiourea **7a-d**. **5a-d** reacts with thionyl chloride in pyridine to give 4-hydroxy-3-(6-thioxo-5,6-dihydro[1,2,4]triazolo[5,1-*b*][1,3,4]oxadiazol-2-yl)-2*H*[1]-benzopyran-2-one **6a-d**. **7a-d** on treatment with ethanol and iodine yields 4-hydroxy-3-[6-phenylimino-6*H*-[1,2,4]-thiadiazolo[3,2-*b*][1,3,4]-oxadiazol-2-yl]-2*H*[1]benzopyran-2-one **8a-d**.

Keywords: 3-formyl 4-hydroxycoumarin, semicarbazide, regioselective condensation, phenyl isothiocynate

IPC: Int.Cl.[8]C07D

Benzopyrans are known to show antifungal[1], anti-coagulant[2], antibacterial[3], and insecticidal[4] activity. The biological importance and considerable therapeutic potential of 3-substituted-4-hydroxybenzopyrans generated interest in designing the synthesis of a number of 3-substituted-4-hydroxybenzopyrans which might be potential candidates as HIV protease inhibitors with a high therapeutic index[5]. 1,3,4-Oxadiazole, 1,3,5-triazine and 1,2,4-triazolothiadiazoles are known to show pesticidal and herbicidal activity. Therefore, the synthesis of 4-hydroxybenzopyranes having these moieties attached at the 3-position was undertaken in order to get molecules with enhanced biological activity.

3-Formyl-4-hydroxycoumarin[6] was treated with semicarbazide in presence of sodium acetate to give 4-hydroxy-2-oxo-2*H*[1]benzopyran-3-aldehyde semicarbazone **1a-d**. On treatment with bromine and sodium acetate, **1a-d** gave 3-(5-amino-1,3,4-oxadiazol-2-yl)-4-hydroxy-2*H*[1]-benzopyran-2-one **2a-d**. The compounds **2a-d** on reaction with benzaldehyde gave 4-hydroxy-3-(5-benzylidineimino1,3,4-oxadiazol-2-yl)-2*H*[1]-benzopyran-2-ones **3a-d**. On cyclisation with phenyl isothiocynate, **3a-d** yielded 3-(6,7-diphenyl-5-thioxo-6,7-dihydro-5*H*-[1, 3, 4]oxadiazolo[3,2-*a*][1,3,5]triazin-2-yl)-4-hydroxy-2*H*[1]-benzopyran-2-ones **4a-d**. On refluxing with KSCN in methanol and phenyl isothiocynate in acetic acid separately, **2a-d** gave N-[5-(4-hydroxy-2-oxo-2*H*[1]-benzopyran-3-yl)-1, 3, 4, -oxadiazol-2-yl]thiourea **5a-d** and N-[5-(-4-hydroxy-2-oxo-2*H*[1]benzopyran-3-yl)-1, 3, 4-oxadiazol-2-yl]-N′-phenylthiourea **7a-d** respectively. The compounds **5a-d** underwent cyclisation in presence of thionyl chloride and pyridine to give 4-hydroxy-3-(6-thioxo-5,6-dihydro[1, 2, 4]triazolo[5, 1-*b*][1, 3, 4]oxadiazol-2-yl)-2*H*[1]-benzopyran-2-one **6a-d**. Whereas, in iodine solution **7a-d** underwent cyclisation to give 4-hydroxy-3-[6-phenylimino-6*H*-[1, 2, 4]-thiadiazolo[3, 2-*b*][1, 3, 4]-oxadiazol-2-yl]-2*H*[1] benzopyran-2-one **8a-d** (**Scheme I**). The structures of the above compounds were in agreement with spectral and analytical data (**Table I**) and they were screened for antibacterial activity against a number of bacterial strains (**Table II**).



1-8a :R_1,R_2,R_3=H
1-8b :R_1=CH_3; R_2,R_3=H
1-8c :R_1,R_3=H; R_2=CH_3
1-8d :R_1,R_2 =H; R_3 =CH_3

Scheme I

Table I—Characterization data of compounds **1a-d**, **2a-d**, **3a-d**, **4a-d**, **5a-d**, **6a-d**, **7a-d** and **8a-d**

Compd	Mol. formula	R_1	R_2	R_3	m.p. °C	Yield (%)	Found (Calcd)% C	H	N	S
1a	$C_{11}H_9O_4N_3$	H	H	H	265	73	53.42 (53.44	3.69 3.64	17.02 17.00	-)
1b	$C_{12}H_{11}O_4N_3$	CH_3	H	H	263	75	55.15 (55.17	4.25 4.21	16.04 16.09	-)

—Contd

Table I—Characterization data of compounds **1a-d**, **2a-d**, **3a-d**, **4a-d**, **5a-d**, **6a-d**, **7a-d** and **8a-d**.**—***Contd*

Compd	Mol. formula	R_1	R_2	R_3	m.p. °C	Yield (%)	Found (Calcd)% C	H	N	S
1c	$C_{12}H_{11}O_4N_3$	H	CH_3	H	193	72	55.16	4.26	16.11	
							(55.17	4.21	16.09	-)
1d	$C_{12}H_{11}O_4N_3$	H	H	CH_3	215	74	55.19	4.22	16.06	
							(55.17	4.21	16.09	-)
2a	$C_{11}H_7O_4N_3$	H	H	H	207	63	53.89	2.82	17.08	
							(53.88	2.86	17.14	-)
2b	$C_{12}H_9O_4N_3$	CH_3	H	H	216	60	55.56	3.43	16.28	-)
							(55.60	3.48	16.22	
2c	$C_{12}H_9O_4N_3$	H	CH_3	H	223	64	55.57	3.50	16.20	
							(55.60	3.48	16.22	-)
2d	$C_{12}H_9O_4N_3$	H	H	CH_3	214	59	55.63	3.47	16.18	
							(55.60	3.48	16.22	-)
3a	$C_{18}H_{11}O_4N_3$	H	H	H	235	68	64.74	3.25	12.63	
							(64.87	3.30	12.61	-)
3b	$C_{19}H_{13}O_4N_3$	CH_3	H	H	243	53	65.59	3.78	12.07	
							(65.71	3.75	12.10	-)
3c	$C_{19}H_{13}O_4N_3$	H	CH_3	H	241	57	65.70	3.73	12.13	
							(65.71	3.75	12.10	-)
3d	$C_{19}H_{13}O_4N_3$	H	H	CH_3	249	61	65.68	3.70	12.11	
							(65.71	3.75	12.10	-)
4a	$C_{25}H_{16}O_4N_4S$	H	H	H	262	72	64.14	3.45	12.01	6.79
							(64.10	3.42	11.97	6.84)
4b	$C_{26}H_{18}O_4N_4S$	CH_3	H	H	256	70	64.70	3.71	11.58	6.71
							(64.73	3.73	11.62	6.64)
4c	$C_{26}H_{18}O_4N_4S$	H	CH_3	H	259	73	64.63	3.77	11.53	6.58
							(64.73	3.73	11.62	6.64)
4d	$C_{26}H_{18}O_4N_4S$	H	H	CH_3	254	67	64.68	3.70	11.56	6.62
							(64.73	3.73	11.62	6.64)
5a	$C_{12}H_8O_4N_4S$	H	H	H	239	65	47.32	2.60	18.39	10.47
							(47.37	2.63	18.42	10.53)
5b	$C_{13}H_{10}O_4N_4S$	CH_3	H	H	240	66	48.98	3.12	17.58	10.02
							(49.06	3.14	17.61	10.06)
5c	$C_{13}H_{10}O_4N_4S$	H	CH_3	H	236	60	49.03	3.11	17.60	10.00
							(49.06	3.14	17.61	10.06)
5d	$C_{13}H_{10}O_4N_4S$	H	H	CH_3	251	67	49.01	3.13	17.60	10.00
							(49.06	3.14	17.61	10.06)
6a	$C_{12}H_6O_4N_4S$	H	H	H	289	73	47.59	1.91	18.52	10.55
							(47.68	1.99	18.54	10.59)
6b	$C_{13}H_8O_4N_4S$	CH_3	H	H	294	69	49.34	2.51	17.70	10.11
							(49.36	2.53	17.72	10.12)
6c	$C_{13}H_8O_4N_4S$	H	CH_3	H	278	72	49.35	2.50	17.68	10.09
							(49.36	2.53	17.72	10.12)
6d	$C_{13}H_8O_4N_4S$	H	H	CH_3	264	77	49.34	2.49	17.70	10.13
							(49.36	2.53	17.72	10.12)
7a	$C_{18}H_{12}O_4N_4S$	H	H	H	268	58	56.82	3.13	14.71	8.40
							(56.84	3.16	14.74	8.42)
7b	$C_{19}H_{14}O_4N_4S$	CH_3	H	H	253	52	57.81	3.52	14.19	8.08
							(57.87	3.55	14.21	8.12)
7c	$C_{19}H_{14}O_4N_4S$	H	CH_3	H	258	59	57.83	3.51	14.19	8.12
							(57.87	3.55	14.21	8.12)
7d	$C_{19}H_{14}O_4N_4S$	H	H	CH_3	272	60	57.82	3.54	14.20	8.10
							(57.87	3.55	14.21	8.12)
8a	$C_{18}H_{10}O_4N_4S$	H	H	H	280	48	57.13	2.64	14.80	8.45
							(57.14	2.65	14.81	8.47)
8b	$C_{19}H_{12}O_4N_4S$	CH_3	H	H	282	51	58.10	3.00	14.22	8.12
							(58.16	3.06	14.28	8.16)
8c	$C_{19}H_{12}O_4N_4S$	H	CH_3	H	277	59	58.13	3.01	14.23	8.14
							(58.16	3.06	14.28	8.16)
8d	$C_{19}H_{12}O_4N_4S$	H	H	CH_3	273	50	58.12	3.05	14.27	8.11
							(58.16	3.06	14.28	8.16)

Antibacterial Activity

All the above synthesized compounds were screened *in vitro* for their antibacterial activity against a variety of bacterial strains. Gram negative strain of bacteria used were *S. typhi* and *E. coli* while gram positive bacterial strain used was *S. aureus*. The minimum inhibitory concentration (MIC) was determined using Tube Dilution technique according to standard procedure[7] (**Table II**). The standard drugs used for comparison were ciprofloxacin, cloxacillin and gentamycin. By careful study of the antibacterial activity data it can be observed that most of the compounds possess significant antibacterial activity.

Experimental Section

Melting points were determined 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 spectra in DMSO-d_6 were recorded on Varian VXR-300 NMR spectrometer using TMS as internal standard and mass spectra were recorded on Shimadzu GC-MS. The homogeneity of the compounds was established by TLC on silica gel plates. The spots were visualised in iodine vapor.

General procedure for the synthesis of 4-hydroxy-2-oxo-2*H*[1]-benzopyran-3-aldehyde semicarbazone 1a-d. To an ethanolic solution of 3-formyl-4-hydroxycoumarin (0.01 mole), an ethanolic solution of semicarbazide (0.01 mole) and sodium acetate (0.01 mole) was added. The reaction mixture was stirred at rt for 3 hr and then refluxed for 1 hr. A solid separated which was filtered, washed with water and purified by recrystallisation from ethanol to obtain **1a-d**.

General procedure for the synthesis of 3-(5-amino-1, 3, 4-oxadiazol-2-yl)-4-hydroxy-2*H*[1]-benzopyran-2-one 2a-d. Bromine (0.8 mL) in acetic acid (5 mL) was added to a stirred slurry of semicarbazone **1a-d** (0.005 mole) and anhydrous sodium acetate (6 g) in acetic acid (17.5 mL). The mixture was stirred at rt for 2 hr. It was poured into water to obtain **2a-d**. The solid obtained was filtered, washed with water, dried and purified by recrystallisation from chloroform.

General procedure for the synthesis of 4-hydroxy-3-(5-benzylidine imino 1,3,4-oxadiazol-2-yl)-2*H*[1]-benzopyran-2-one 3a-d. A mixture of oxadiazole **2a-d** (0.01 mole) and benzaldehyde (0.01mole) in absolute ethanol (25 mL) was refluxed for 4 hr and filtered while hot. On cooling the filtrate the solid obtained was filtered and purified by recrystallisation from ethanol.

General procedure for the synthesis of 3-(6,7-diphenyl-5-thioxo-6,7-dihydro-5*H*-[1,3,4]oxadiazolo[3, 2-a][1, 3, 5]triazin-2-yl)-4-hydroxy-2*H*[1]-benz`opyran-2-one 4a-d. A mixture of **3a-d** (0.002

Table II − Antibacterial activity of compounds **1a-d**, **2a-d**, **3a-d**, **4a-d**, **5a-d, 6a-d, 7a-d** and **8a-d**

Compd	Antibacterial activity(µg/mL)			Compd	Antibacterial activity(µg/mL)		
	S.aureus	*S.typhi*	*E.coli*		*S.aureus*	*S.typhi*	*E.coli*
1a	-	-	-	**5a**	-	150	145
1b	-	-	-	**5b**	-	-	150
1c	-	-	-	**5c**	-	-	140
1d	-	-	-	**5d**	140	100	95
2a	-	-	-	**6a**	85	90	110
2b	-	150	150	**6b**	90	120	100
2c	-	-	-	**6c**	80	80	65
2d	-	150	150	**6d**	95	70	80
3a	145	130	95	**7a**	100	115	85
3b	150	145	100	**7b**	90	120	80
3c	90	125	95	**7c**	85	85	90
3d	80	80	105	**7d**	50	70	65
4a	145	130	95	**8a**	100	115	85
4b	150	145	100	**8b**	90	120	80
4c	90	125	95	**8c**	85	85	90
4d	80	80	105	**8d**	50	70	65

——=Not active up to 150 µg/mL

mole) and phenylisothiocynate (0.002 mole) was refluxed for 7 hr in dry toluene.The solvent was distilled off under reduced pressure. The residue obtained was filtered, washed with a small amount of ethanol followed by water and purified by recrystallisation from ethanol.

General procedure for the synthesis of N-[5-(4-hydroxy-2-oxo-2*H*[1]-benzopyran-3-yl)-1,3,4,-oxadiazol-2-yl]thiourea 5a-d. A mixture of oxadiazole **2a-d** (0.01 mole) and KSCN (0.01 mole) was refluxed in methanol for 4 hr to give **5a-d**. The reaction mixture was poured into crushed ice, filtered and the solid product was purified by recrystallisation from ethanol.

General procedure for the synthesis of 4-hydroxy-3-(6-thioxo-5,6-dihydro[1,2,4]triazolo[5,1-b][1,3,4]oxadiazol-2-yl)-2*H*[1]-benzopyran-2-one 6a-d. A mixture of **5a-d** (0.02 mole) and thionyl chloride (0.025 mole) was refluxed in pyridine (20 mL) for 6 hr and the solvent evaporated. The concentrate was poured into ice-cold water. The residue was washed with water and purified by recrystallisation from ethanol to give **6a-d**.

General procedure for the synthesis of N-[5-(4-hydroxy-2-oxo-2*H*[1]benzopyran-3-yl)-1,3,4-oxadiazole-2-yl]-N′-phenylthiourea 7a-d. Oxadiazole **2a-d** (0.01mole) and phenyl isothiocyanate were refluxed in acetic acid for 10 hr and then poured into crushed ice, filtered, washed with water and purified by recrystallisation from acetic acid.

General procedure for the synthesis of 4-hydroxy-3-[6-phenylimino-6*H*-[1, 2, 4]-thiadiazolo-[3,2-b][1,3,4]-oxadiazol-2-yl]-2*H*[1]benzopyran-2-one 8a-d. A solution of **7a-d** (0.02 mole) in ethanol was treated with a solution of iodine in EtOH-H_2O (80:20; v/v) till decolourisation of iodine was no longer observed. It was then refluxed for 1 hr and then cooled to rt. On addition of NH_4OH the desired product precipitated out. It was filtered and purified by recrystallisation from ethanol to give **8a-d**.

1c: IR (KBr): 3424(OH, NH_2), 2928(CH str.), 1723(>C=O), 1669, 1611, 1376 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.20(s, 3H, CH_3), 5.10(s, 1H, CH=N), 7.20-7.70(m, 3H, CH_3 aromatic H), 7.75(s, 2H, NH_2, D_2O exchangeable), 7.95(s, 1H, NH, D_2O exchangeable), 9.80(s, 1H, OH, D_2O exchangeable).

2c: IR (KBr): 3444(OH, NH_2), 2359, 1733(>C=O), 1619, 1448, 1393 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.2(s, 3H, CH_3), 5.00(s, 2H, NH_2, D_2O exchangeable), 7.5(d, 1H, C_6'-H, *J*=7.5 Hz), 7.7(d, 1H, C_5'-H, *J*=7.5 Hz) 8.1(s, 1H, C_8'-H),10.18(s, 1H, OH, D_2O exchangeable).

3c: IR (KBr): 3420(OH), 2924, 1731(>C=O), 1659, 1620, 1447, 1384 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.22(s, 3H, CH_3), 5.78(s, 1H, N=CH), 7.2(m, 5H, aromatic-H), 7.53(d, 1H, C_6'-H, *J*=7.0 Hz), 7.79(d, 1H, C_5'-H, *J*=7.0 Hz) 8.0(s, 1H, C_8'-H),10.28(s, 1H, OH, D_2O exchangeable).

4c: IR (KBr): 3278(OH), 2923, 2086, 1729(>C=O), 1659, 1618, 1443, 1378, 1319, 1176, 1047, 757 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.31(s, 3H, CH_3), 4.00(s, 1H, N-CH-N), 6.9-7.1(m, 10H, aromatic-H), 7.49(d, 1H, C_6'-H, *J*=8.0 Hz), 7.78(d, 1H, C_5'-H, *J*=8.0 Hz), 7.98(s, 1H, C_8'-H), 10.00(s, 1H, OH, D_2O exchangeable); MS: m/z (%) 482(M^+), 481, 392, 378, 175, 148, 147, 135, 134, 119, 106, 91, 78.

5c: IR (KBr): 3440(OH, NH, NH_2), 2925, 1732(>C=O), 1658, 1620, 1448, 1385, 1315, 1228, 1176, 1057, 788 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.1(s, 3H, CH_3), 6.25(s, 2H, NH_2, D_2O exchangeable), 7.19(s, 1H, NH, D_2O exchangeable), 7.52(d, 1H, C_6'-H, *J*=7.5 Hz), 7.70(d, 1H, C_5'-H, *J*=7.5 Hz), 7.90(s, 1H, C_8'-H), 10.23(s, 1H, OH, D_2O exchangeable).

6c: IR (KBr): 3424(OH, NH), 1727(>C=O), 1656, 1619, 1383, 1315, 1176, 1055, 787 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.0(s, 3H, CH_3), 5.5(s, 1H, NH, D_2O exchangeable), 7.6(d, 1H, C_6'-H, *J*=7.5 Hz), 7.8(d, 1H, C_5'-H, *J*=7.5 Hz), 8.19(s, 1H, C_8'-H), 10.00(s, 1H, OH, D_2O exchangeable); MS: m/z (%) 316(M^+), 219, 217, 203, 201, 187, 182, 175, 174, 148, 135, 134, 119, 106, 99, 78, 72, 45, 28, 27.

7c: IR (KBr): 3438(OH, NH), 2923, 1730(>C=O), 1659, 1619, 1444, 1383, 1315, 1231, 1174, 1055, 786 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.22(s, 3H, CH_3), 6.6 and 7.1 (s, 2H, NH, D_2O exchangeable), 6.9-7.0(m, 5H, aromatic-H), 7.5(d, 1H, C_6'-H, *J*=8.0 Hz), 7.7(d, 1H, C_5'-H, *J*=8.0 Hz), 8.0(s, 1H, C_8'-H), 10.4(s, 1H, OH, D_2O exchangeable).

8c: IR (KBr): 3120(-OH), 2358, 1730(>C=O), 1659, 1618, 1539, 1450, 1314, 1232, 1174, 1055, 976 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.21(s, 3H, CH_3), 7.39-7.41(m, 5H, aromatic-H), 7.6(d, 1H, C_6'-H, *J*=8.0 Hz), 7.88(d, 1H, C_5'-H, *J*=8.0 Hz), 8.2(s, 1H, C_8'-H), 10.22(s, 1H, OH, D_2O exchangeable); MS: m/z (%) 392(M^+), 219, 217, 203, 201, 187, 175, 174, 148, 147, 135, 134, 131, 119, 106, 104, 91, 78, 72.

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References

1 Sangwan N K, Verma B S, Malik O P & Dhindsa K S, *Indian J Chem*, 29B, **1990**, 294.

2 Stahman M A, Huebner C F & Link K P, *J Biol Chem*, 138, **1941**, 513.

3 Honmantgad S S, Kulkarni M V & Patil V D, *Indian J Chem*, 24B, **1985**, 459.

4 Hapworth J D, *Comprehensive Heterocyclic Chemistry*, 3 rd edition. Edited by J A Boultonand and A Mikillap, (Pergamon Press, Oxford), **1984**, 737.

5 Mitra J & Mitra A K, *Indian J Chem*, 35B, **1996**, 588.

6 Mulwad V V & Shirodkar J M, *J Heterocyclic Chem*, 40, **2003**, 377.

7 Frankle S, Reitman S & Sonnenwirth A C, *Gradwol's Clinical Laboratory Method and Diagnosis*, 7th edition, Vol 2, (C V Mosby Co, Germany), **1970**, 1406.