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Note

Synthesis and antibacterial activity of 6-(5-phenyl-[1,3,4]thiadiazol-2-ylamino)-benzopyran-2-ones

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6-Amino coumarins **1a-c** on treatment with carbon disulphide in presence of iodine in pyridine give 6-isothiocyanato coumarins **2a-c**. Condensation of the acid hydrazide with 6-isothiocyanato coumarins has resulted in the formation of 2-benzoyl-*N*-(2-oxo-2*H*-benzopyran-6-yl)hydrazinecarbothioamides **3a-f** which are cyclized to 6-(5-phenyl-[1,3,4]thiadiazol-2-ylamino)-benzopyran-2-ones **4a-f** using Conc. H_2SO_4. The structures of the compounds **2a-c**, **3a-f** and **4a-f** have been established on the basis of spectral and analytical data. All compounds have been screened for their antimicrobial activity and showed that the introduction of 1,3,4-thiadiazole show significant antibacterial activities.

Keywords: 6-Isothiocyanato coumarins, 1,3,4-thiadiazole, iodine, pyridine, acid hydrazide

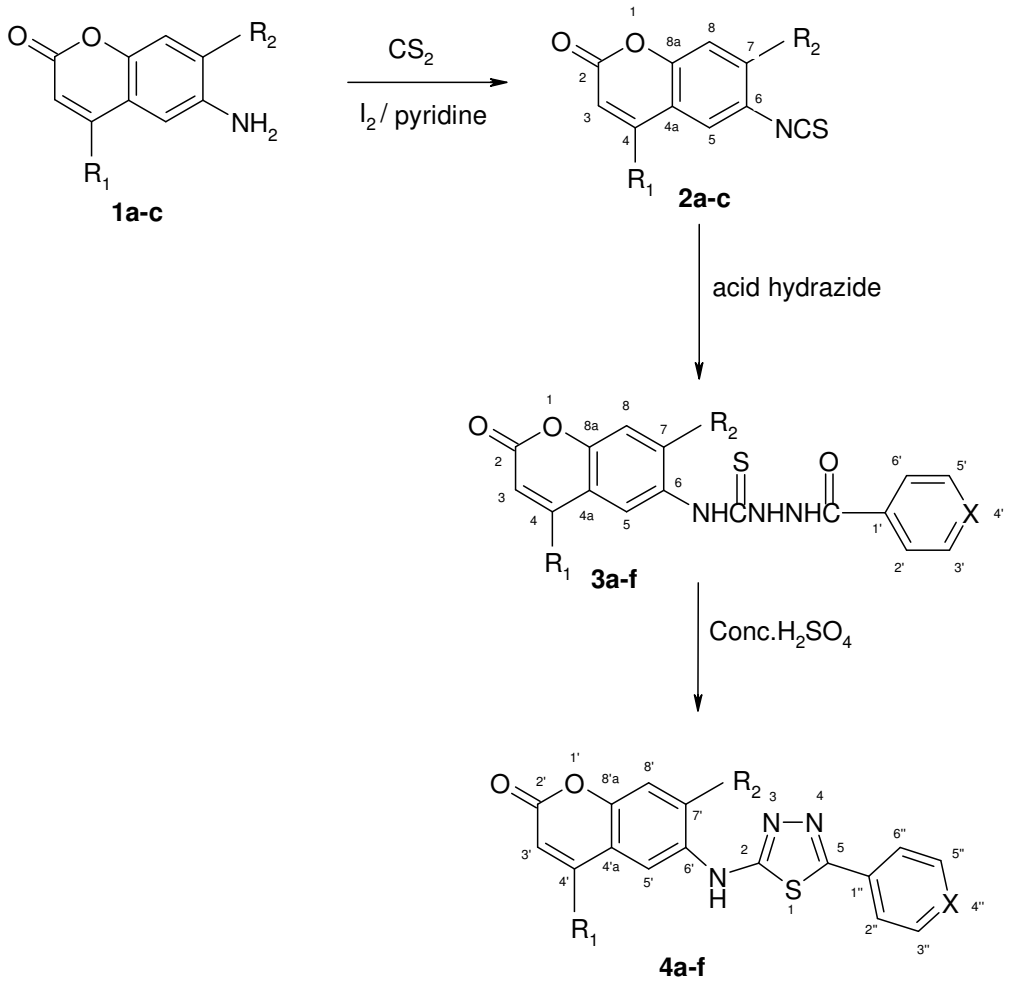
During recent years there has been intense investigation of different classes of thiadiazole compounds, many of which are known to possess interesting biological properties such as anti-microbial[1-3], antituberculosis[4], antiinflammatory[5-7], anticonvulsant[8,9], antihypertensive[10,11], local anesthetic[12], anticancer[13,14], and hypoglycemic activities[15]. In addition, 1,3,4-thiadiazoles exhibit various biological activities possibly due to the presence of the –N=C-S moiety[16]. Nitrogen mustards synthesized from 6-aminocoumarins exhibit carcinogenic activity[17]. They are also known to possess antiviral[18] activity and especially effective against HIV_1(ref. 19). The Schiff bases of 6-aminocoumarins have been reported to exhibit biological activities like antibacterial and antifungal[20] activities. The biological importance of 1,3,4-thiadiazole derivatives has prompted us to synthesize 1,3,4-thiadiazoles derived from 6-amino coumarins, which may have some of the biological activity.

Results and Discussion

For this purpose, 6-isothiocyanato coumarins **2a-c** being the starting material were prepared from 6-amino coumarins **1a-c** treatment with carbon disulphide and iodine in pyridine. Structures **2a-c** were confirmed on the basis of spectral and analytical data. The IR spectrum of **2a** in KBr showed presence of bands at 2079 for –NCS and 1726 cm^{-1} for >C=O of coumarin. The ^1H NMR spectrum of the same in $CDCl_3$ showed a singlet at δ 2.48 for three protons of methyl group at C_7. Doublet observed at δ 6.41 and 7.60 for C_3-H and C_4-H respectively at J = 9Hz. Singlet observed at δ 7.19 and 7.33 for C_8-H and C_5-H proton respectively. The ^{13}C NMR spectrum showed signal at δ 18.9 for the methyl carbon at C_7, 160.0 for the carbonyl of coumarin ring and other signal at 152.3, 142.0, 139.6, 136.8, 127.2, 124.4, 118.7, 117.5, 117.1. The condensation of the acid hydrazide with 6-isothiocyanato coumarins resulted in the formation of 2-benzoyl-*N*-(2-oxo-2*H*-benzopyran-6-yl)hydrazinec-arbothioamides **3a-f**. Compounds **3a-f** are cyclized to 6-(5-phenyl-[1,3,4]thiadiazol-2-ylamino)-benzopyran-2-ones **4a-f (Scheme I)** using Conc. H_2SO_4 as dehydrating agent. The IR spectrum of **4a** in KBr showed presence of bands at 3331 for NH stretching, 1716 cm^{-1} for >C=O of coumarin. The ^1H NMR spectrum of the same in DMSO-d_6 showed a singlet at δ 2.39 for three protons of methyl group at $C_{7'}$. Doublet observed at 6.42 and 8.04 for $C_{3'}$-H and $C_{4'}$-H respectively at J = 9Hz. Singlet observed at 7.34 for $C_{8'}$-H proton. The multiplet were observed at δ 7.46-7.55 for three protons at $C_{3''}$, $C_{5''}$ and $C_{4''}$-H. Doublet observed at δ 7.82 for two protons of $C_{2''}$ and $C_{6''}$-H. Singlet at δ 8.28 for one proton of $C_{5'}$-H and broad singlet observed at 9.77 was observed for one proton of NH which is D_2O exchangeable. The disappearance of other two NH group proved the product formation. The ^{13}C NMR spectrum of compound **4a** in DMSO-d_6 showed signal at δ 18.4 for one CH_3 carbon at $C_{7'}$. Signal at δ 160.2 for >C=O of coumarin, signal at δ 165.9 and 158.1 for C_2 and C_5 of thiadiazole respectively and other signal at 149.8, 144.2, 139.7, 134.9, 129.3, 128.8, 126.7, 120.0, 118.3, 118.0, 117.1, 115.7. The disappearance of >C=S signal also proved the product formation.

Antimicrobial activity

All the synthesized compounds **2a-c**, **3a-f** and **4a-f** were screened for their antibacterial activity against



1a, **2a**:	R_1=H,	R_2=CH_3	**3a**,**4a**:	R_1=H,	R_2=CH_3,	X=CH
1b, **2b**:	R_1=CH_3,	R_2=CH_3	**3b**,**4b**:	R_1=CH_3,	R_2=CH_3,	X=CH
1c, **2c**:	R_1=H,	R_2=H	**3c**,**4c**:	R_1=H,	R_2=H,	X=CH
			3d,**4d**:	R_1=H,	R_2=CH_3,	X=N
			3e,**4e**:	R_1=CH_3,	R_2=CH_3,	X=N
			3f,**4f**:	R_1=H,	R_2=H,	X=N

Scheme I

S. aureus, *S. typhi* and *E. coli* (**Table I**) by the drug diffusion method[21]. The zone of inhibition was measured in mm and was compared with standard drug. DMSO was used as a blank and streptomycin was used as antibacterial standard. All the compounds were tested at 100 µg/mL and 250 µg/mL concentration.

From the antimicrobial screening of the compounds it could observe that the introduction of 1,3,4-thiadiazole show significant antibacterial activities, also compound **3a-f** show comparable antibacterial activity.

Experimental Section

General: Melting points were taken in open capillaries and are uncorrected. Purity of the compounds was checked on TLC. IR spectra (ν in cm^{-1}) were recorded on a Perkin-Elmer FTIR, ^{1}H NMR on a 300 MHz JEOL NMR AL300 using TMS as standard and mass spectra on a Shimadzu GC-MS QP-2010. All the compounds gave satisfactory elemental analysis.

General procedure for the synthesis of 6-isothiocyanato coumarins 2a-c

A solution of 2.54 g of iodine (10 mmole) in 20 mL of carbon disulphide was added drop-wise to a

Table I — Antibacterial activity of compounds **2a-c**, **3a-f** and **4a-f**

Compd	Zone of inhibition in mm					
	S. aureus		*S. typhi*		*E. coli*	
	100µg	250µg	100µg	250µg	100µg	250µg
2a	-	9	11	12	12	13
2b	-	10	12	13	12	14
2c	-	9	-	10	-	11
3a	-	11	11	13	14	15
3b	12	13	14	15	14	16
3c	-	11	11	13	12	13
3d	11	12	13	15	16	17
3e	13	14	12	14	13	15
3f	11	12	13	14	12	14
4a	16	18	17	19	17	20
4b	17	19	15	16	18	20
4c	14	15	15	17	15	17
4d	15	16	18	20	18	20
4e	16	18	17	21	18	20
4f	15	17	14	16	15	18

Disc size: 6.35mm Duration: 24 hrs. Standard: streptomycin resistant (11mm/less) Control: DMSO intermediate (12-14mm) sensitive (15mm/more)

suspension of 1.89 g of 6-amino coumarin (10 mmole) in 20 mL of pyridine at 0°C. The contents were stirred for 4.0 h at 0°C. The reaction-mixture was distilled until all traces of carbon disulphide and pyridine were removed. Residue was treated with excess of dil. HCl and the separated solid was filtered, dried and further purified by using silica gel column chromatography eluting with hexane and EtOAc.

2a: Mol. Formula $C_{11}H_7NO_2S$, m.p. 192-94, yield: 89%; IR (KBr): 3076, 3030, 2957 (-CH), 2079 (-NCS), 1726 (>C=O), 1624, 1550, 1131, 1103, 887, 836 cm^{-1}; ^1H NMR ($CDCl_3$, δ): 2.48 (s, 3H, C_7-CH_3), 6.41 (d, 1H, *J* = 9Hz, C_3-H), 7.19 (s, 1H, C_8-H), 7.33 (s, 1H, C_5-H), 7.60 (d, 1H, *J* = 9Hz, C_4-H); ^{13}C NMR ($CDCl_3$, δ): 18.9, 117.1, 117.5, 118.7, 124.4, 127.2, 136.8, 139.6, 142.0, 152.3, 160.0; Mass (*m/z* %): M^+ 217 (100), 189 (37), 160 (40), 131 (22), 117 (15), 102 (42), 89 (27), 77 (75), 63 (48).

2b: Mol. Formula $C_{12}H_9NO_2S$, m.p. 230-32, yield: 86%; IR (KBr): 3082, 3046, 2962 (-CH), 2114 (-NCS), 1734 (>C=O), 1620, 1261, 1095, 1024, 801 cm^{-1}; ^1H NMR ($CDCl_3$, δ): 2.33 (s, 3H, C_4-CH_3), 2.40 (s, 3H, C_7-CH_3), 6.22 (s, 1H, C_3-H), 7.11 (s, 1H, C_8-H), 7.33 (s, 1H, C_5-H); ^{13}C NMR ($CDCl_3$, δ): 18.6, 18.8, 115.5, 118.8, 118.9, 121.6, 127.1, 136.5, 139.5, 151.2, 151.8, 160.1; Mass (*m/z* %): M^+ 231 (100), 202 (44), 188 (10), 170 (18), 145 (15), 115 (21), 102 (8).

2c: Mol. Formula $C_{10}H_5NO_2S$, m.p. 184-86, yield: 88%; IR (KBr): 3085, 3063 (-CH), 2084 (-NCS), 1721 (>C=O), 1618, 1562, 1179, 1106, 888, 821 cm^{-1}; ^1H NMR ($CDCl_3$, δ): 6.48 (d, 1H, *J* = 9Hz, C_3-H), 7.24-7.40 (m, 3H, C_5, C_7 and C_8-H), 7.64 (d, 1H, *J* = 9Hz, C_4-H); Mass (*m/z* %): M^+ 203 (100), 175 (48), 146 (12), 117 (8), 103 (6), 89 (13).

General procedure for the synthesis of 2-benzoyl-*N*-(2-oxo-*2H*-benzopyran-6-yl)hydrazinecarbothioamide 3a-f

To the solution of 7.5 mmole of benzoic acid hydrazide or isoniazid in 30 mL of ethanol was added 7.5 mmole of the appropriate 6-isothiocyanato coumarins. The mixture was refluxed on water-bath for 3 h, excess of ethanol was removed by distillation. The solid product obtained on cooling was filtered and washed with water and recrystallized from ethanol.

3a: Mol. Formula $C_{18}H_{15}N_3O_3S$, m.p. 116-20, yield: 79%; IR (KBr): 3238 (-NH), 3061, 2968 (-CH), 1717, 1680 (>C=O), 1627, 1522, 1240, 1128, 1098, 828 cm^{-1}; ^1H NMR (DMSO-d_6, δ): 2.26 (s, 3H, C_7-CH_3), 6.39 (d, 1H, *J* = 9Hz, C_3-H), 7.29 (s, 1H, C_8-H), 7.44-7.60 (m, 4H, C_5, $C_{3'}$, $C_{5'}$ and $C_{4'}$-H), 7.95 (d, 2H, *J* = 6Hz, $C_{2'}$ and $C_{6'}$-H), 8.05 (d, 1H, *J* = 9Hz, C_4-H), 9.63 (s, 1H, NH-exchangeable), 9.76 (s, 1H, NH-exchangeable), 10.57 (s, 1H, NH-exchangeable); ^{13}C NMR (DMSO-d_6, δ): 18.5, 115.9, 117.0, 118.3, 126.5, 127.3, 128.7, 132.3, 133.4, 143.2, 145.6, 152.4, 155.2, 160.8, 160.9, 181.2.

3b: Mol. Formula $C_{19}H_{17}N_3O_3S$, m.p. 173-75, yield: 80%; IR (KBr): 3258 (-NH), 3061, 2978 (-CH), 1702, 1685 (>C=O), 1624, 1524, 1249, 1160, 1051, 699 cm^{-1}; ^1H NMR (DMSO-d_6, δ): 2.38 (s, 6H, C_4, C_7-

CH_3), 6.23 (s, 1H, C_3-H), 7.21 (s, 1H, C_8-H), 7.48 (t, 2H, J = 9Hz, $C_{3'}$ & $C_{5'}$-H), 7.60 (t, 1H, J = 9Hz, $C_{4'}$-H), 7.76 (s, 1H, C_5-H), 7.80 (d, 2H, J = 9Hz, $C_{2'}$ and $C_{6'}$-H), 9.68 (s, 1H, NH-exchangeable), 9.82 (s, 1H, NH-exchangeable), 10.62 (s, 1H, NH-exchangeable).

3c: Mol. Formula $C_{17}H_{13}N_3O_3S$, m.p. 125-27, yield: 82%; IR (KBr): 3258 (-NH), 3054, 2969 (-CH), 1727, 1690 (>C=O), 1629, 1529, 1255, 1168, 1041, 692 cm^{-1}; ^1H NMR (DMSO-d_6, δ): 6.48 (d, 1H, J = 9Hz, C_3-H), 7.34 (d, 1H, J = 9Hz, C_8-H), 7.49 (t, 2H, J = 6Hz, $C_{3'}$ and $C_{5'}$-H), 7.60-7.86 (m, 5H, C_7, C_5, $C_{2'}$, $C_{6'}$ and $C_{4'}$-H), 8.07 (d, 1H, J = 9Hz, C_4-H), 9.60 (s, 1H, NH-exchangeable), 9.74 (s, 1H, NH-exchangeable), 10.66 (s, 1H, NH-exchangeable).

3d: Mol. Formula $C_{17}H_{14}N_4O_3S$, m.p. 174-76, yield: 77%; IR (KBr): 3301, 3233 (-NH), 3047, 2978 (-CH), 1734,1680 (>C=O), 1626, 1533, 1265, 1173, 1044, 798 cm^{-1}; ^1H NMR (DMSO-d_6, δ): 2.24 (s, 3H, C_7-CH_3), 6.41 (d, 1H, J = 9Hz, C_3-H), 7.29 (s, 1H, C_8-H), 7.66 (s, 1H, C_5-H), 7.84 (d, 2H, J = 6Hz, $C_{2'}$ and $C_{6'}$-H), 8.08 (d, 1H, J = 9Hz, C_4-H), 8.75 (d, 2H, J = 6Hz, $C_{3'}$ and $C_{5'}$-H), 9.95 (br, 2H, two NH-exchangeable), 10.89 (s, 1H, NH-exchangeable).

3e: Mol. Formula $C_{18}H_{16}N_4O_3S$, m.p. 189-91, yield: 79%; IR (KBr): 3307, 3228 (-NH), 2964 (-CH), 1715, 1678 (>C=O), 1624, 1560, 1261, 1095, 1022, 801 cm^{-1}; ^1H NMR (DMSO-d_6, δ): 2.37 (s, 6H, C_4, C_7-CH_3), 6.23 (s, 1H, C_3-H), 7.21 (s, 1H, C_8-H), 7.75 (s, 1H, C_5-H), 7.82 (d, 2H, J = 6Hz, $C_{2'}$ and $C_{6'}$-H), 8.74 (d, 2H, J = 6Hz, $C_{3'}$ and $C_{5'}$-H), 9.84 (s, 1H, NH-exchangeable), 9.95 (s, 1H, NH-exchangeable), 10.88 (s, 1H, NH-exchangeable).

3f: Mol. Formula $C_{16}H_{12}N_4O_3S$, m.p. 163-65, yield: 80%; IR (KBr): 3289, 3206 (-NH), 3082, 3030 (-CH), 1748, 1678 (>C=O), 1645, 1574, 1544, 1214, 820 cm^{-1}; ^1H NMR (DMSO-d_6, δ): 6.47 (d, 1H, J = 9Hz, C_3-H), 7.36 (d, 1H, J = 9Hz, C_8-H), 7.60 (d, 1H, J = 9Hz, C_7-H), 7.72 (s, 1H, C_5-H), 7.84 (d, 2H, J = 6Hz, $C_{2'}$ and $C_{6'}$-H), 8.07 (d, 1H, J = 9Hz, C_4-H), 8.76 (d, 2H, J = 6Hz, $C_{3'}$ and $C_{5'}$-H), 9.99 (br, 2H, two NH-exchangeable), 10.90 (s, 1H, NH-exchangeable).

General procedure for the synthesis of 6-(5-phenyl-[1,3,4]thiadiazol-2-ylamino)-benzopyran-2-ones 4a-f

To 1 mmole of appropriate acid carbazide was added concentrated sulphuric acid (1 mL) drop-wise. The mixture was stirred at room temperature for 3 h. The reaction-mixture was poured into ice-water mixture. The solid product obtained was filtered and washed with aqueous sodium carbonate solution followed by water and recrystallized from ethanol.

4a: Mol. Formula $C_{18}H_{13}N_3O_2S$, m.p. 196-98, yield: 74%; IR (KBr): 3331 (-NH), 3040 (-CH), 1716 (>C=O), 1630, 1563, 1497, 1420, 1119, 750 cm^{-1}; ^1H NMR (DMSO-d_6, δ): 2.39 (s, 3H, $C_{7'}$-CH_3), 6.42 (d, 1H, J = 9Hz, $C_{3'}$-H), 7.34 (s, 1H, $C_{8'}$-H), 7.48 (m, 3H, $C_{3''}$, $C_{5''}$ and $C_{4''}$-H), 7.82 (d, 2H, J = 6Hz, $C_{2''}$ and $C_{6''}$-H), 8.04 (d, 1H, J = 9Hz, $C_{4'}$-H), 8.28 (s, 1H, $C_{5'}$-H), 9.77 (s, 1H, NH-exchangeable); ^{13}C NMR (DMSO-d_6, δ): 18.4, 115.7, 117.1, 118.0, 118.3, 120.0, 126.7, 128.8, 129.3, 134.9, 139.7, 144.2, 149.8, 158.1, 160.2, 165.9.

4b: Mol. Formula $C_{19}H_{15}N_3O_2S$, m.p. 243-46, yield: 76%; IR (KBr): 3310 (-NH), 3050, 2978 (-CH), 1699 (>C=O), 1624, 1569, 1493, 1420, 1384, 1263, 1181, 1054, 886, 753, 684 cm^{-1}; ^1H NMR (DMSO-d_6, δ): 2.40 (s, 6H, $C_{4'}$, $C_{7'}$-CH_3), 6.37 (s, 1H, $C_{3'}$-H), 7.33 (s, 1H, $C_{8'}$-H), 7.48 (m, 3H, $C_{3''}$, $C_{5''}$and $C_{4''}$-H), 7.83 (d, 2H, J = 9Hz, $C_{2''}$ and $C_{6''}$-H), 8.36 (s, 1H, $C_{5'}$-H), 9.79 (s, 1H, NH-exchangeable); Mass (*m/z* %): M^+ 349 (100), 334 (12), 316 (18), 246 (50), 231 (21), 213 (29), 189 (32), 160 (22), 118 (23), 103 (21), 91 (16), 77 (24).

4c: Mol. Formula $C_{17}H_{11}N_3O_2S$, m.p. 185-87, yield: 75%; IR (KBr): 3321 (-NH), 3046, 2976 (-CH), 1690 (>C=O), 1628, 1578, 1502, 1429, 1367, 1255, 1171, 1040, 888, 745, 678 cm^{-1}; ^1H NMR (DMSO-d_6, δ): 6.46 (d, 1H, J = 9Hz, $C_{3'}$-H), 7.40 (d, 1H, J = 9Hz, $C_{8'}$-H), 7.48 (m, 3H, $C_{3''}$, $C_{5''}$ and $C_{4''}$-H), 7.60 (d, 1H, J = 9Hz, $C_{7'}$-H), 7.83 (d, 2H, J = 6Hz, $C_{2''}$ & $C_{6''}$-H), 8.08 (d, 1H, J = 9Hz, $C_{4'}$-H), 8.24 (s, 1H, $C_{5'}$-H), 9.76 (s, 1H, NH-exchangeable).

4d: Mol. Formula $C_{17}H_{12}N_4O_2S$, m.p. 234-36, yield: 68%; IR (KBr): 3311 (-NH), 3055, 2960 (-CH), 1696 (>C=O), 1622, 1567, 1486, 1420, 1368, 1245, 1163, 1054, 871, 765, 660 cm^{-1}; ^1H NMR (DMSO-d_6, δ): 2.37 (s, 3H, $C_{7'}$-CH_3), 6.41 (d, 1H, J = 9Hz, $C_{3'}$-H), 7.34 (s, 1H, $C_{8'}$-H), 7.73 (d, 2H, J = 6Hz, $C_{2''}$ and $C_{6''}$-H), 8.05 (d, 1H, J = 9Hz, $C_{4'}$-H), 8.27 (s, 1H, $C_{5'}$-H), 8.75 (d, 2H, J = 6Hz, $C_{3''}$ and $C_{5''}$-H), 9.82 (s, 1H, NH-exchangeable).

4e: Mol. Formula $C_{18}H_{14}N_4O_2S$, m.p. 255-57, yield: 71%; IR (KBr): 3297 (-NH), 3049, 2970 (-CH), 1700 (>C=O), 1633, 1549, 1488, 1428, 1364, 1244, 1180, 1025, 876, 755, 679 cm^{-1}; ^1H NMR (DMSO-d_6, δ): 2.38 (s, 6H, $C_{4'}$, $C_{7'}$-CH_3), 6.35 (s, 1H, $C_{3'}$-H), 7.32 (s, 1H, $C_{8'}$-H), 7.74 (d, 2H, J = 6Hz, $C_{2''}$ and $C_{6''}$-H), 8.35 (s,1H, $C_{5'}$-H), 8.75 (d, 2H, J = 6Hz, $C_{3''}$ and $C_{5''}$-H), 9.80 (s, 1H, NH-exchangeable).

4f: Mol. Formula $C_{16}H_{10}N_4O_2S$, m.p. 219-21, yield: 70%; IR (KBr): 3311 (-NH), 3059, 2969 (-CH), 1696 (>C=O), 1646, 1585, 1497, 1433, 1345, 1225, 1146, 1036, 868, 735, 669 cm^{-1}; ^1H NMR (DMSO-d_6, δ): 6.45 (d, 1H, J = 9Hz, $C_{3'}$-H), 7.40 (d, 1H, J = 9Hz, $C_{8'}$-H), 7.60 (d, 1H, J = 9Hz, $C_{7'}$-H), 7.75 (d, 2H, J = 6Hz, $C_{2''}$ and $C_{6''}$-H), 8.08 (d, 1H, J = 9Hz, $C_{4'}$-H), 8.23 (s, 1H, $C_{5'}$-H), 8.74 (d, 2H, J = 6Hz, $C_{3''}$ and $C_{5''}$-H), 9.83 (s, 1H, NH-exchangeable).

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