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One Pot Synthesis Of Pyrimidine And Bispyrimidine Derivatives And Their Evaluation For Anti-Microbial And Anti-Oxidant Activities

Varsha Samdhian[1], Ramandeep Kaur[2], Balbir Kaur[1], Swaranjit Singh Cameotra[3]

1. Department of Chemistry, Punjabi University, Patiala-147002, **INDIA**
2. Department of Chemistry, S.U.S. Govt. College, Sunam-148028, **INDIA**
3. Institute of Microbial Technology, Chandigarh-160036, **INDIA**

Email: drbalbir@gmail.com

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ABSTRACT

The heterocyclic compounds are of great importance to life as their structural subunits exist in many natural products such as vitamins, hormones, pigments etc. Modern society is dependent on synthetic heterocycles for many uses such as drugs, pesticides, plastics, dyes, cosmetics, information storage, solvents, antioxidants, vulcanization accelerators and in rubber industry. Keeping this in view, different bis pyrimidines were synthesized. These were further screened for antimicrobial and anti-oxidant activities.

Keywords: Heterocycles, bis pyrimidines, antimicrobial, antioxidant activities.

INTRODUCTION

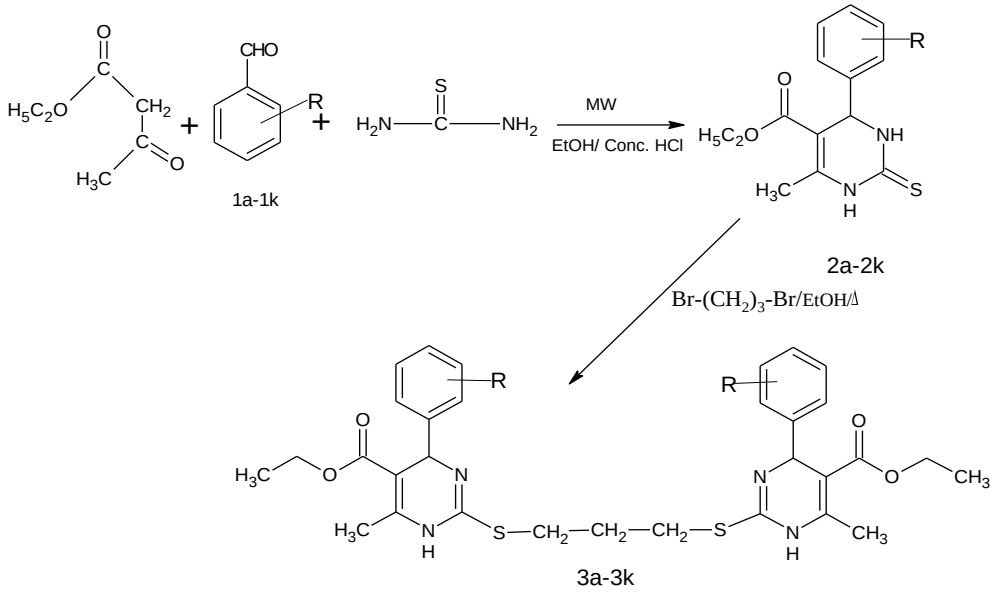
Pyrimidine moiety is an important class of nitrogen containing heterocycles [1] and is widely used as a key building block for pharmaceutical agents. Its derivatives exhibit antibacterial, antifungal [2], analgesic [3], calcium antagonist [4], anti-inflammatory [5] and anti-tumor activity [6]. In 1893, Biginelli reported one-step reaction for synthesis of 3,4-dihydropyrimidine-2-(1H)-one by three-component reaction of ethylacetoacetate, urea and benzaldehyde [7]. In continuous quest of more pharmacological activities, we herein report the synthesis of certain bis pyrimidine compounds.

MATERIALS AND METHODS

A mixture of thiourea (.01 mol, 0.8g), ethylacetoacetate (.01 mol,1.30g) , substituted aromatic aldehydes (.01 mol) and 4-5 drops of conc. HCl was taken into beaker and small amount of absolute alcohol added. The reaction mixture was irradiated in the microwave at 320 W. Further, it was kept overnight undisturbed. After that, alcohol was added to obtain the solid.

General Procedure of bis-1,4-dihydropyrimidines: 1g of THP is dissolved in absolute alcohol, after complete dissolution, ½ mol of dibromopropane was added and reaction mixture was refluxed for some hours till the completion of reaction. These structures were confirmed through their PMR and mass spectral studies. The ultraviolet spectra of these did not show any bathochromic shift which would have

been possible if the two double bonds were conjugated. Thus the structure with two non-conjugated double bonds is suggested in Scheme 1



R: a = H; b = OCH_3; c = 3-NO_2; d = 4-NO_2; e = 2-NO_2; f = 4-OH; g = 2-OH; h = 4-OH, 3-OCH_3; i = 2,3-(OCH_2O); j = 2,4-$(Cl)_2$; k = 3,4 $(OCH_3)_2$

Scheme 1. Synthesis of bispyrimidines

RESULTS AND DISCUSSION

Characterization of compounds (2a-2k):

S.No.	R	M.pt(^{0}C)	Time(min)	Colour
2a	H	203-204	10	White
2b	4-OCH_3	137-138	6	Light yellow
2c	3-NO_2	195-199	4	white
2d	4-NO_2	175-177	5	yellow
2e	2-NO_2	218-220	5	yellow
2f	4-OH	178-180	5	yellow
2g	2-OH	155-157	6	white
2h	4-OH,2-OCH_3	213-215	8	yellow
2i	2,3-(OCH_2O)	165-167	4	light yellow
2j	2,4-$(Cl)_2$	105-107	7	white
2k	3,4-$(OCH_3)_2$	149-150	4	yellow

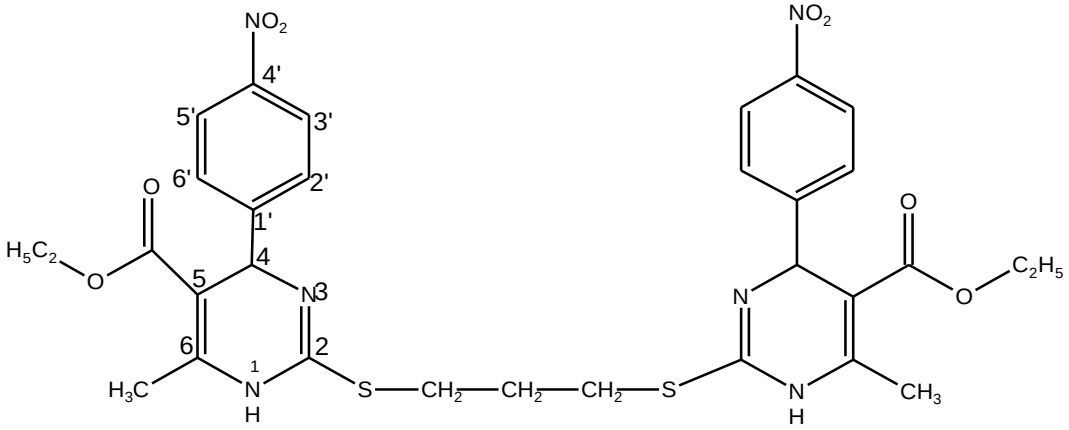
In all of these compounds (2a-2k), PMR spectra is completely similar except for multiplicity of aromatic protons which obviously depends upon the substitution pattern in the ring. In all these compounds there is triplet and quartet for ester function and a singlet for 6-CH_3 protons almost without any shift when compared with each other. However, as expected the position of N-H proton did vary in its chemical shift but could be traced easily through its disappearance on D_2O exchange. These compounds were converted to their bis-pyrimidines (3a-3k).

Spectral features of some compounds:
2a : ^{1}H-NMR (400 MHz,$CDCl_3$):δ:10.05 & 9.43 (s, 1H, NH), 7.32-7.22 (m, H, Ar-H), 4.07-4.02 (q, 2H, O-$\underline{CH_2}$-CH_3), 2.33 (s, 3H, C_6CH_3), 1.17-1.12 (t, 2H, O-CH_2-$\underline{CH_3}$) ; IR (KBr) cm^{-1}:3175 (sec. N-H str.), 2979 (asymmetric C-H str.), 2935 (symmetric C-H str.), 1670 (C=O str. of ester), 1574 (C=C skeletal vib. of aromatic ring), 1465 (C=C str.), 1176 (C-N vib.), 1118 (C=S str.)

Characterization of compounds (3a-3k)

S.No.	R	M.pt(^{0}C)	Colour
3a	H	167-169	yellow
3b	4-OCH_3	73-75	yellow
3c	3-NO_2	82-83	yellow
3d	4-NO_2	71-72	yellow
3e	2-NO_2	112-113	yellow
3f	4-OH	120-122	yellow
3g	2-OH	70-72	white
3h	4-OH,2-OCH_3	73-75	yellow
3i	2,3-(OCH_2O)	85-87	yellow
3j	2,4-$(Cl)_2$	145-147	white
3k	3,4-$(OCH_3)_2$	106-108	brown

3a : Yield (0.5 g, 30%); white solid; m.p. 170-173°C; IR (KBr): υ_{max} (cm^{-1}) 3329 (N-H), 3070 (aromatic C-H), 2979, 2899 (methylene C-H), 1670 (C=O), 1574 (C=N); ^{1}H-NMR (400 MHz,$CDCl_3$): δ 9.57 (2H, s, N-H), 7.50-7.42 (10H, m, H-2'-6'), 5.47 (2H, s, H-4), 4.22 (4H, q, O-C***H***$_2$-CH_3), 3.77 (4H, t, S-C***H***$_2$-CH_2-), 2.53 (6H, s, CH_3-6), 1.37 (2H, m, S-CH_2-C***H***$_2$), 1.33 (6H, t, O-CH_2-C***H***$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$): 174.17 (C=O), 165.07 (C-2), 144.37 (C-6), 143.25 (C-1'), 127.94 (C-5'), 127.82 (C-6'), 127.18 (C-3'), 126.31 (C-2'), 126.15 (C-4'), 100.92 (C-5), 59.28 (O***C***H_2), 54.37 (C-4), 39.93 (S-***C***H_2-), 38.88 (S-***C***H_2-***C***H_2-), 17.15 (***C***H_3-6), 13.67 (O-CH_2-***C***H_3). mass (m/z): 593 (100%), 533, 460, 427



diethyl 2,2'-(propane-1,3-diylbis(sulfanediyl))bis(6-methyl-4-(4-nitrophenyl)-1,4-dihydropyrimidine-5-carboxylate)
Chemical Formula: $C_{31}H_{34}N_6O_8S_2$

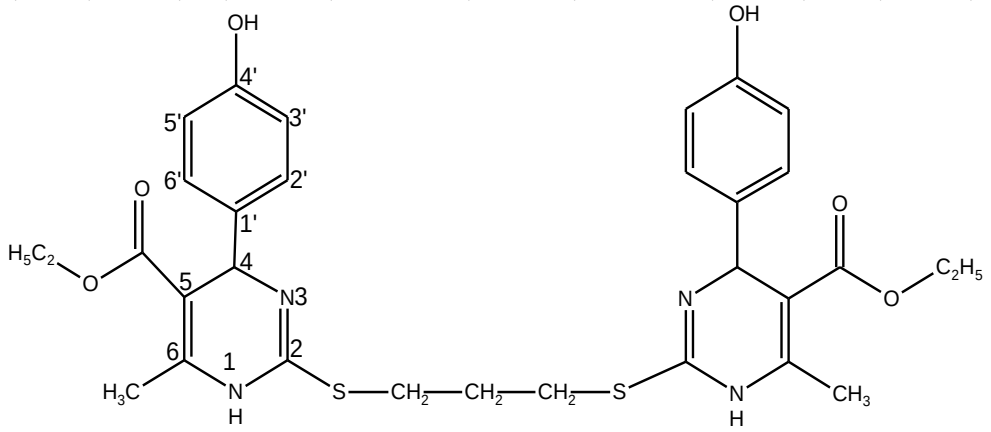
Exact Mass: 682.19

Molecular Weight: 682.77

m/z: 682.19 (100.0%), 683.19 (35.8%), 684.18 (9.1%), 684.19 (8.4%), 685.19 (3.4%), 683.18 (2.2%), 685.20 (1.2%)

Elemental Analysis: C, 54.53; H, 5.02; N, 12.31; O, 18.75; S, 9.39

3d : IR (KBr): υ_{max} (cm^{-1}) 3186 (N-H), 3112 (aromatic C-H), 2977, 2933 (methylene C-H), 1699 (C=O), 1645 (C=N); ^{1}H-NMR (400MHz, DMSO): δ 10.4 (1H, s, NH-1/NH-1a), 9.69 (1H, s, NH-1/NH-1a), 8.26-8.14 (4H, d, H-3', 5', 3a'and 5a', J_o=8.6 Hz), 7.73-7.46 (4H, d, H-2', 6', 2a'and 6a', J_o=8.6 Hz), 5.79 (1H, d, H-4/H-4a), 5.30 (1H, d, H-4/H-4a), 4.07-3.94 (4H, q, (CH_3-C***H***$_2$-O)$_2$), 3.87 (1H, m, S-C***H***$_2$-), 2.50 (1H, m, S-C***H***$_2$), 2.30 (3H, s, CH_3-6/CH_3-6a), 2.26 (3H, s, CH_3-6/CH_3-6a), 2.10 (2H, t, S-CH_2-CH_2-C***H***$_2$-S), 1.21-1.07 (2H, m, S-CH_2-C***H***$_2$-), 0.82-0.78 (6H, t, C***H***$_3$-CH_2-O); ^{13}C-NMR (100MHz, DMSO-**d_6**): δ 174.47 (C=O), 164.7 (C-6), 161.1 (C-2), 149.2 (C-1'), 144.9 (C-4'), 129.1 (C-2',6'), 123.9 (C-3', 5'), 103.52 (C-5), 62.72 (OCH_2), 59.6 (C-4), 26.38 (S-***C***H_2-CH_2), 21.79 (S-CH_2-***C***H_2), 17.2 (CH_3-6), 13.9 (***C***H_3-CH_2-O).



diethyl 2,2'-(propane-1,3-diylbis(sulfanediyl))bis(4-(4-hydroxyphenyl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate)

Chemical Formula: $C_{31}H_{36}N_4O_6S_2$

Exact Mass: 624.21

Molecular Weight: 624.77

m/z: 624.21 (100.0%), 625.21 (35.8%), 626.20 (9.1%), 626.21 (7.7%), 627.21 (3.3%), 625.20 (1.5%), 627.22 (1.0%)

Elemental Analysis: C, 59.59; H, 5.81; N, 8.97; O, 15.37; S, 10.26

3f

IR **:** 3275 (O-H Str), 3175 (N-H Str), 2962 (aromatic C-H), 1678 (C=O Str of ester), 1457 (C=C), 1238 (C-N Vib); ^{1}H-NMR (400MHz, DMSO): δ 10.1 (1H, s, NH-1/NH-1a), 9.61 (1H, s, OH-4'/OH-4a'), 9.27 (1H, s, OH-4'/OH-4a'), 9.46 (1H, s, NH-1/NH-1a), 7.05-6.99 (4H, d, H-2', 6', 2a'and 6a'), 6.78-6.64 (4H, d, H-3', 5', 3a'and 5a'), 5.18 (1H, d, H-4/H-4a), 5.07 (1H, d, H-4/H-4a), 4.20-3.90 (4H, q, (CH_3-C***H***$_2$-O)$_2$), 3.29 (2H, t, S-C***H***$_2$-), 3.22 (1H, t, S-C***H***$_2$), 2.35 (3H, s, CH_3-6/CH_3-6a), 2.30 (3H, s, CH_3-6/CH_3-6a), 1.19-0.82 (8H, m, S-CH_2-C***H***$_2$- & (C***H***$_3$-CH_2-O)$_2$); ^{13}C-NMR (100MHz, DMSO): δ 173.8 (C=O), 165.1 (C-OH), 156.8 (C-6), 144.26 (C-2), 134.1 (C-1'), 128.8 (C-2',6'), 115.1 (C-3', 5'), 101.16 (C-5), 59.2 (OCH_2), 53.6 (C-4), 26.0 (S-***C***H_2-CH_2), 22.1 (S-CH_2-***C***H_2), 17.1 (CH_3-6), 13.9 (***C***H_3-CH_2-O).

3i

^{1}H-NMR (400MHz, DMSO): δ 7.9 (2H, s, N-H), 7.0-6.6 (6H, m, H-4', 5', 6', 4a', 5', 6a'), 5.97 (4H, s, OCH_2O), 5.32 (2H, H-4 & H-4a), 4.18 (4H, q, (CH_3-C***H***$_2$-O)$_2$), 3.67 (4H, t, S-C***H***$_2$), 2.37 (6H, s, CH_3-6), 1.29-1.17 (8H, m, S-CH_2-C***H***$_2$- & (C***H***$_3$-CH_2-O)$_2$); ^{13}C-NMR (100MHz, DMSO): 163.98 (C=O), 161.0 (C-6), 149.09 (C-2), 142.61 (C-2'), 142.36 (C-3'), 136.48 (C-1'), 121.71 (C-5'), 120.41 (C-6'), 108.84 (C-4'), 102.95 (C-5), 101.84 (OCH_2O), 61.12 (OCH_2), 55.98 (C-4), 26.62 (S-***C***H_2), 25.0 (S-CH_2-***C***H_2), 18.41 (CH_3-6), 14.14 (***C***H_3-CH_2-O).

3k

^{1}H-NMR (400MHz, DMSO): δ 7.99 (2H, s, N-H), 7.28 (2H, d, H-2', J_o=8.6 Hz), 7.17 (2H, d, H-5', J_o=8.6 Hz), 6.65 (2H, d, H-6', J_o=8.6 Hz), 5.31 (2H, d, H-4), 4.09 (4H, q, CH_3-C***H***$_2$-O), 2.99 (12H, s, OCH_3), 2.89 (4H, t, S-CH_2), 2.36 (6H, s, CH_3-6), 1.34 (2H, m, S-CH_2-CH_2), 1.22 (6H, t, CH_3-CH_2-O).

APPLICATIONS

Antimicrobial assays: Screening of compounds for antibacterial/antifungal activity was done by Agar well diffusion method (Table 1). A lawn of culture was prepared by spreading the 100µl culture broth having 106 CFU mL-1 of test organism on agar plates. Plates were left standing for 10-15 min to let the culture absorbed. 8 mm size wells were punched into the agar with the help of sterile micropipette tips. Wells were sealed with one drop of molten agar (0.8% nutrient agar) to prevent leakage of compound from the bottom of the plate. The wells were filled with 100µL of test compound (concentration 100µg mL-1, prepared in 1% DMSO). Solvent blank was used as negative control. Plates were incubated at 370C for 24 h. The antibacterial activity was evaluated by measuring the zone of inhibition (tables 1 and2).

Table 1: Antimicrobial activity of chemical compounds using agar well diffusion method.

Test compounds	Zone of inhibition (mm)																	
	Strains tested																	
	B. subtilis			*E.coli* MTCC131			*P. aeruginosa*			*S. aureus* MTCC3160			*C. albicans*			*C.albicans* MTCC186		
Concentration (µg/ml)	100	400	1000	100	400	1000	100	400	1000	100	400	1000	100	400	1000	100	400	1000
3a	-	10	13	-	-	-	9	12	22	-	-	11	-	-	-	-	-	13
3b	-	-	-	-	-	-	13	14	20	-	-	-	-	-	-	-	-	14
3c	-	-	12	-	-	-	14	15	15	-	-	9	-	-	-	-	10	19
3d	-	10	11	-	-	11	9	10	17	-	-	12	-	-	--	-	-	14
3e	-	-	10	-	-	13	16	16	17	-	-	10	-	-	-	-	-	18
3f	-	-	12	-	-	9	9	11	21	-	-	-	-	-	--	-	-	18
3g	-	-	17	-	-	-	12	12	15	-	-	-	-	-	-	-	-	19
3h	-	-	9	-	-	-	17	17	19	-	-	-	-	-	-	-	-	19
3i	-	-	9	-	-	-	14	14	20	-	-	11	-	-	-	-	11	22
3j	-	-	12	-	9	12	-	-	20	-	-	13	13	14	17	9	12	14
3k	-	-	15	-	-	-	-	-	17	-	-	12	18	18	20	10	12	22
DMSO (solvent control)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2: Antimicrobial activity of antibiotics using agar well diffusion method.

Test Compounds Concentration (100 µg/ml)	Zone of inhibition (mm)					
	Strains tested					
	B. subtilis	*E. coli* MTCC131	*P. aeruginosa*	*S. aureus* MTCC3160	*C. albicans*	*C. albicans* MTCC186
Amoxycillin	28	15	17	31	ND	ND
Ciprofloxacin	46	36	38	38	ND	ND
Fluconazole	ND	ND	ND	ND	22	18

Method for *In Vitro* Antioxidant Activity

DPPH Radical Scavenging Assay: Free radical scavenging activity (Table 3) of different fractions against stable DPPH (1, 1, Diphenyl-2- picryl-hydrazyl) was determined spectrophotometrically by the modified method of Gyamfi et al [8]. When DPPH reacts with an antioxidant, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were measured at 517 nm on a UV-Vis spectrophotometer (Spectronic 20 D+, USA). A 50 μL of the test compounds raging from 5 to 2000 μg mL^{-1} was mixed with 1 ml of 0.1 mM DPPH in methanol solution and 450 μL of 50 mM Tris-HCl buffer (pH 7.4). Absorbance of the DPPH radical without antioxidant, *i.e.* the control, was measured at 517 nm. Methanol was used as a solvent blank in the experiment. After 1 hr of incubation under dark at room temperature, the reduction of the DPPH free radicals was measured spectrophotometrically at 517 nm. Ascorbic acid was used as positive control. Percent inhibition of the DPPH radical by the samples was calculated according to the formula of Yen & Duh [9].

$$\% \text{ inhibition} = ((A_{C(o)} - A_{A(t)}) / A_{C(o)}) \times 100$$

Where $A_{C(o)}$ is the absorbance of the control at t = 0 min and $A_{A(t)}$ is the absorbance of the antioxidant at t = 1 h.

Table 3: Antioxidant activities of compounds

Test compounds	% Inhibition of scavenging activity								EC_{50}*
	Concentration (µg/ml)								
	2000	1000	500	250	100	50	25	5	
3a	4.76	4.63	2.64	0	0	0	0	0	
3b	59.86	16.11	5.42	3.78	2.96	0	0	0	
3c	58.88	17.76	4.60	3.12	2.96	1.31	0.65	0	
3d	65.46	48.19	27.63	22.69	4.44	1.31	0.98	0.16	
3e	67.10	65.13	44.07	33.88	4.69	1.31	0.98	0	
3f	95.55	93.42	90.95	80.26	79.11	69.07	58.38	51.15	
3g	46.54	45.55	26.80	9.53	2.96	0.98	0	0	
3h	90.62	88.98	80.09	58.05	35.85	5.75	0.98	0	
3i	96.38	83.88	80.26	59.70	35.03	22.53	4.60	0	
3j	82.73	80.26	72.03	40.62	33.88	14.30	2.96	0	
3h	96.54	96.05	84.37	68.42	43.91	27.46	12.00	1.31	
(Control) Ascorbic acid	83.71	78.45	67.10	61.84	43.42	40.62	34.37	32.39	

The data highlighted in yellow are negative for antioxidant activity, in blue are moderate antioxidant, and in green are strong antioxidant including ascorbic acid (Observation is based on change of violet colour to pale yellow colour instantly).* The effective concentration of sample required to scavenge DPPH radical by 50% (EC_{50}) value is to be obtained for each sample by linear regression analysis of dose response curve by plotting between % inhibition and concentrations of compounds.

CONCLUSIONS

The newly synthesized compounds were screened for their antimicrobial activity. The compounds (3a-3i) showed very good antibacterial activity whereas the compound 3j and 3k were completely inactive against antibacterial strain i.e. Pseudomonas aeruginosa, as representative of gram negative strain. The compound with functional group R=4-OH, 3-OCH_3 (3h) possessed antibacterial activity equal to that of reference drug. The antibacterial data of present study indicated that gram negative bacterial strain is most sensitive against compounds under investigation. This could be explained by the presence of lypophilic moieties in the bis-dihydropyrimidine scaffold.

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