Research Article

Synthesis, Antioxidant and Antimicrobial Activity of New Series of 3-Substituted Chromen-4-One Derivatives

S. Ushasri[1]*, B. Sarvani[1], J. Ranjith Kumar[2] and CH. Sudha Bhargavi[2]

[1]Hindu College of Pharmacy, Amaravati Road, Guntur-522002, Andhra Pradesh, India.
[2]Sri Siddhartha Pharmacy College, Ammavarithota, Nuzvid-521201, Andhra Pradesh, India.

ABSTRACT
A new series of chromen-4-one derivatives were synthesized by utilising the Vilsmeier Hack[7] reaction. Initially 2-hydroxy acetophenone was condensed with DMF and phosphorus oxy chloride to yield chromone-3-carbaldehyde. The reaction of 3-carbaldehyde derivative with phenyl hydrazine HCl and thioglycollic acid, gave 3-thiazolidinyl chromones. The same reaction using chloro acetyl chloride instead of thioglycollic acid yielded 3-azetidinyl chromones. Further, the above -3-cabaldehyde mediated reactions were modified to form 3-carbamyl chromones and 3-thio carbamyl chromones by replacing DMF with urea and thiourea respectively. The synthesised compounds were purified by column chromatography using solvents like n-hexane, alcohol and chloroform. The structures of these compounds were established on the basis of spectral and elemental analysis. The compounds were evaluated for their anti oxidant and anti microbial activity. As a result of this screening, some compounds have shown the promising anti oxidant activity and excellent antimicrobial activity, when compared with standards.

Keywords: chromen-4-one derivatives, Vilsmeier Hack reaction, anti oxidant activity.

INTRODUCTION

A number of natural and synthetic benzopyrone derivatives have been reported to exert notable antimicrobial[1], antifungal[2] and antioxidant activity[3,8]. Benzopyrones having chromone (γ-benzopyrone) moiety are associated with interesting physiological activities such as antimicrobial[9], antitubercular[10], anti-inflammatory[5], antidiabetic, antiviral[6], anticancer[4] etc. In view of these observations, our interest is to synthesise a new series of chromen-4-one derivatives. For this 3-carbaldehyde and 3-carbamyl derivatives were prepared from 2-hydroxy acetophenone and the condensation of the above derivatives with chloroacetyl chloride and thio glycollic acid, in presence of HCl, gave the final compounds, 3-azetidinyl chromones and 3-thiazolidinyl chromones respectively. And these derivatives were screened for antimicrobial and antioxidant activity.

MATERIALS AND METHODS

Synthetic starting material, reagents, and solvents were of analytical grade or of the highest quality commercially available. The chemicals were purchased from Avra Synthesis Pvt Ltd, Hyderabad and Merck Chemical Co., Germany respectively, and were dried whenever necessary. The Melting points of the synthesised compounds were determined by open capillary method and are uncorrected. Purity of the compounds was verified on TLC plates coated with silica gel-G using ethyl acetate and benzene in 1:1 ratio as mobile phase. IR spectra were recorded on thermo Nicolet IR 200 spectrometer using KBr disc method. H^1NMR spectra were recorded on BRUKER amx-400 NMR spectrometer where $CDCl_3$ is used as internal standard. Results of Combustion analysis were found to be within the limits of permissible errors.

Anti oxidant activity

Oxidative stress was found to be a major contributor for numerous patho physiological conditions including cancer. Therefore oxidative damage can prevent this risk effectively. Anti oxidant efficiency of compounds was verified by using nitric oxide generated due to decomposition of sodium nitro prusside in acqeous medium which combines with oxygen to produce nitrite ions. These are measured by Gries' reaction. Later the nitrite ions are subjected to azo dye coupling and absorbance is measured at 546 nm. Another method employed is, scavenging of super oxide radical with alkaline DMSO

using nitroblue tetrazolium and measuring the absorbance against blank at 560 nm.

Antimicrobial Activity

The synthesised compounds were screened for antimicrobial activity against gram-positive bacteria like Bacillus pumilus, Bacillus subtilis and gram negative bacteria like Escherichia coli and Proteus vulgaris. Similarly the compounds were screened for antifungal activity against fungi like Aspergillus niger and Candida albicans by using cup-borer method. Ciprofloxacin and Micanazole nitrate were used as standard drugs. DMSO was used as solvent.

EXPERIMENTAL

Synthetic procedures for the preparation of compounds (B_1-B_2): Step1

2.7mL of 2-hydroxy acetophenone or 7.6g of 2,4-dihydroxy acetophenone was taken in clean beaker, and is kept in ice bath to maintain 0^0C. Then 11.1mL of DMF was added, maintaining the temperature at 0^0C. To the above reaction mixture 15.5mL of phosphorous oxychloride was added drop wise with vigorous stirring, while temperature is maintained at 0^0C throughout the addition. The reaction mixture was kept aside for 2hrs in cold condition with occasional stirring. To this 25g of crushed ice is added and the precipitated product was recrystallised from ethanol.

Step2; 0.5g of phenyl hydrazine hydrochloride was dissolved in solution of 0.8g of sodium acetate in 5mL water. To this solution, 0.2g of recrystallised product (from step-1) and 35mL of ethanol was added to form a clear solution, which on heating yields a precipitate. The precipitate was collected and recrystallised from ethanol.

Step3; 20mL of GAA and 12mL of thioglycollic acid were added to about 0.96g of recrystallised product from step2. This solution was refluxed for 3 hrs and the reaction mixture was cooled to room temperature. To this 25g of crushed ice was added and the precipitated product was recrystallised from chloroform.

Synthetic procedures for the preparation of compounds (B_3-B_4)

(B_3-B_4) The step1 and 2 same as above Step3; 0.86ml of glacial acetic acid was taken added to about 0.96g of recrystallised product from (step-3) by placing in ice bath, to maintain a temperature of 0^0C. Then 0.46ml of chloroacetyl chloride was added with vigorous stirring while maintaining the temp at 0^0C. The reaction mixture was kept in cold condition for 3hrs with occasional stirring. To this 25g of crushed ice was added and the precipitated product was recrystallised from chloroform.

Synthetic procedures for the preparation of compounds (B_5-B_6)

2.7ml of 2-hydroxy acetophenone or 3.1g of 2,4-dihydroxy acetophenone was added to 5.4g of urea taken in a beaker placed in ice bath at 0^0C. To this 15.5ml of phosphorous oxy chloride ($POCl_3$) was added drop wise with vigorous stirring. The solution was continuously stirred for 15min after complete addition of $POCl_3$, and is kept aside at 15^0C for 24hours. The precipitate formed was collected and recrystallised from ethanol.

Synthetic procedures for the preparation of compounds (B_7-B_8)

2.7ml of 2-hydroxy acetophenone or 3.1g of 2,4-dihydroxy acetophenone was added to 3.1g thiourea taken in a beaker, placed in ice bath at 0^0C. To this 15.5ml of phosphorous oxychloride ($POCl_3$) was added drop wise with vigorous stirring. The solution was continuously stirred for 15min after complete addition of $POCl_3$, and is kept aside at 15^0C for 24hrs. The precipitate formed was collected and recrystallised from ethanol.

Anti oxidant activity

Oxidative stress was found to be a major contributor for numerous patho physiological conditions including cancer. Therefore oxidative damage can prevent this risk effectively. Anti oxidant efficiency of compounds was verified by using nitric oxide generated due to decomposition of sodium nitro prusside in aqeous medium which combines with oxygen to produce nitrite ions. These are measured by Gries' reaction. Later the nitrite ions are subjected to azo dye coupling and absorbance is measured at 546 nm. Another method employed is, scavenging of super oxide radical with alkaline DMSO using nitroblue tetrazolium and measuring the absorbance against blank at 560 nm.

Method I: Nitric Oxide radical scavenging activity; The nitric oxide radical scavenging activity was measured by using Griess' reagent. 5ml of each synthesised derivative and ascorbic acid (standard) of different concentrations (25-20µg/ml) in standard phosphate buffer solution (P^H 7.4) were incubated with 5ml of sodium nitro prusside solution (5Mm) at 25^0C for 5hrs. Control was prepared without the compound but with equivalent amount of buffer. After incubation,

0.5ml of Griess' reagent [sulphanilamide (1%), ortho phosphoric acid (2%) and napthyl ethylene diamine di hydrochloride (0.1%)] was added and the absorbance was measured at 546nm against the blank (DMSO). From the absorbance, the present scavenging activity was calculated using the same formula as described above. The experiment was performed in triplicate.

Method-2: Scavenging of Superoxide radical with the alkaline DMSO method; The reaction mixture containing 0.1mL of nitroblue tetrazolium (1mg/mL in DMSO) and 0.3mL of synthesized compound or standard in DMSO was added with 1mL of alkaline DMSO (1mL of DMSO containing NaOH 5mM in 0.1mL of water) to give a final volume of 1.4mL and the absorbance was measured at 560nm against the blank (DMSO).

Antimicrobial Activity

All the synthesized compounds were screened for antibacterial and antifungal activities by paper cup-borer method. The antibacterial activity of the compounds were evaluated against four Gram positive bacteria like Bacillus pumillus(NCIM 2327), Bacillus subtilis(NCIM 2063) and gram negative bacteria like Escherichia coli(NCIM 2607) and Proteus vulgaris(NCIM 2027). The antifungal activities of the synthesized compounds were evaluated against two fungi Aspergillus niger(MTCC 281) and Candida albicans(MTCC 227). The observed data on the antimicrobial activity of the synthesized compounds and standard drugs are given in **Table 5 & 6**.

RESULTS

Spectral data

3-(3^1-anilino-4^1-oxo-thiazolidine-2-yl)-4H-chroman-4-one(B_1): mol. formula $C_{18}H_{14}O_3N_2S$, m.p- 98^{0}C, %Yield- 4.61%v/w, Rf-0.69, IR (KBr) cm^{-1} 3408.09(2^0-N-H, str), 1400.53(-C-N-, str), 1709.52(-C=O, str, 5-membered thiazole), 2922.48(-C-H, str), 1293.26(-C-S, str), 1242.45(-C-N-, str, cyclic), ^{1}HNMR δ(ppm) 0.00(s, 1H, -CH- thiazole), 1.257(s, 2H, -CH_2), 3.870(s, 1H, -NH-), 6.931(m, 1H, -8CH-);

3-(3^1-anilino-4^1-oxo-thiazolidine-2-yl)-7hydroxy-4H-chroman-4-one(B_2): mol. formula $C_{18}H_{14}O_4N_2S$, m.p- 115^{0}C, %Yield- 15.49%w/w, Rf-0.51, IR (KBr) cm^{-1} 3398.40(2^0-N-H, str), 3213.06(Ar-OH, str), 1792.61(-C=O, str, 5-membered thiazole), 1289.94(-C-S, str), 1246.54(-C-N-, str, cyclic), ^{1}HNMR δ(ppm) 1.257(s, 2H, -CH_2-), 3.623(s, 1H, -NH-), 4.706(s, 1H, Ar-OH), 7.228 (m, 2H, 2&3-CH-);

3-(3^1-anilino-2^1-oxo-azetidine-4-yl)-4H-chroman-4-one(B_3): mol. formula $C_{18}H_{14}O_3N_2$, m.p- 87^{0}C, %Yield- 9.98%v/w, Rf-0.67, IR (KBr) cm^{-1} 3425.85(2^0-N-H str), 1383.85(-C-N-str), 1620.76(-C=O, str, 4-membered β-lactam), 1539.09(-C=C-N- str), 1234.11(-C-N-, str, cyclic), 1450.07(-N-N-, str), ^{1}HNMR δ(ppm) 1.261(s, 2H, -CH_2- β-lactam), 3.984(s, 1H, -NH-), 6.814(m, 1H, -2CH-), 7.904 (m, 2H, 2&3-CH-);

3-(3^1-anilino-2^1-oxo-azetidine-4-yl)-7hydroxy-4H-chroman-4-one(B_4): mol. formula $C_{18}H_{14}O_4N_2$, m.p- 109^{0}C, %Yield- 15.49%w/w, Rf-0.54, IR (KBr) cm^{-1} 3373.24(2^0-N-H, str), 3179.44(Ar-OH, str), 1618.23(-C=O, str, 4-membered β-lactam), 1546.84(-C=C-N- str), 1383.85(-C-N-, str, cyclic), 1468.12(-N-N-, str), ^{1}HNMR δ(ppm) 1.259(s, 2H, -CH_2-), 3.984(s, 1H, -NH-), 5.103(s, 1H, Ar-OH), 7.343(m, 2H, 2&3-CH-);

2-amino-4-oxo-4H-chroman-3-carboxamide(B_5): mol. formula $C_{10}H_8O_3N_2$, m.p- 254^{0}C, %Yield- 60.32%v/w, Rf-0.53, IR (KBr) cm^{-1} 3437.40(-N-H str), 1668.53(-C=O, str, amide), 1141.51(-C-O-C- cyclic ester), ^{1}HNMR δ(ppm) 3.210(s, 2H, -NH_2-), 6.978(s, 2H, -CO-NH_2-), 7.343 (d, 1H, 5&8-CH-);

2-amino-7hydroxy-4-oxo-4H-chroman-3-carboxamide(B_6): mol. formula $C_{10}H_8O_4N_2$, m.p- 271^{0}C, %Yield- 74.77%w/w, Rf-0.52, IR (KBr) cm^{-1} 3421.85(Ar-OH, str), 3170.22(-N-H str), 1713.64(-C=O, str, amide), 1241.63(-C-O-C- cyclic ester), ^{1}HNMR δ(ppm) 2.493(s, 2H, -NH_2-), 4.797(s, 1H, Ar-OH), 6.895(s, 2H, -CO-NH_2-), 7.298(d, 1H, 5&8-CH-);

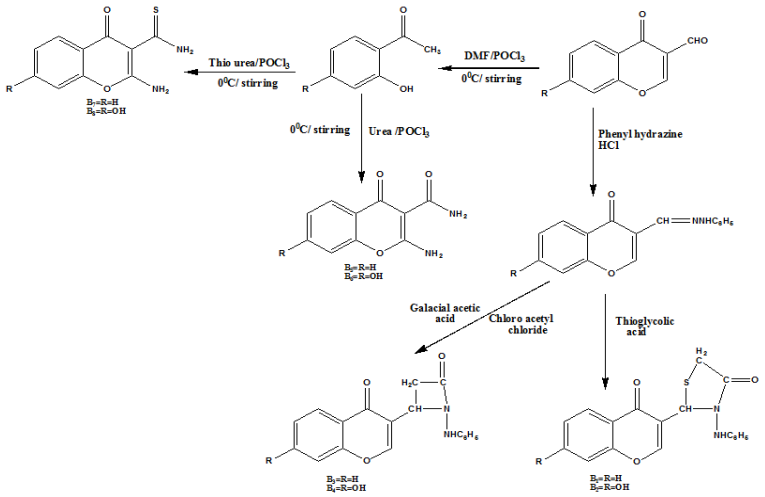
2-amino-4-oxo-4H-chroman-3-carbothioamide(B_7): mol. formula $C_{10}H_8O_2N_2S$, m.p- 101^{0}C, %Yield- 34.42%v/w, Rf-0.51, IR (KBr) cm^{-1} 3284.65(-N-H str), 1086.50(-C-O-C-cyclic ester), 1645.72(-C=O, str, amide), ^{1}HNMR δ(ppm) 2.373(s, 2H, -NH_2-), 6.517(s, 2H, -CO-NH_2-), 6.998(d, 1H, 5&8-CH-);

2-amino-7hydroxy-4-oxo-4H-chroman-3-carbothioamide(B_8): mol. formula $C_{10}H_8O_2N_2S$, m.p- 122^{0}C, %Yield- 50.21%w/w, Rf-0.51, IR (KBr) cm^{-1} 3381.91(Ar-OH, str), 3175.20(-N-H str), 1616.45(-C=O, str, amide), 1082.62(-C-O-C- cyclic ester), ^{1}HNMR δ(ppm) 2.680(s, 2H, -NH_2-), 3.935(s, 1H, Ar-OH), 6.338(s, 2H, -CO-NH_2-), 7.771(d, 1H, 5&8-CH-);

DISCUSSION

In the current study, a series of Chromen-4-one derivatives were synthesized by using Vilsmeier Hack reaction. The compounds were characterized by the spectral methods IR, ^{1}HNMR spectra and their structures were established. When the compounds were evaluated for biological activity, all of them

were effective against both gram positive and gram negative bacteria. The compounds had good antifungal activity against Aspergillus niger but the results were poor against Candida albicans. Compounds from B_1-B_6 exhibited good antioxidant activity.



Scheme 1: Synthetic route for the preparation compounds B_1-B_8

Table 1: Percentage Scavenging Nitric Oxide radical method

Conc (µg/ml)	Standard	Control	B1	B2	B3	B4
0	0	6.78	0	0	0	0
25	23.08	6.78	10.43	10.45	9.21	10.24
50	34.64	6.78	21.4	21.54	16.28	22.13
75	45.17	6.78	29.87	29.89	23.67	30.42
100	59.29	6.78	32.56	39.21	31.34	36.89
125	67.43	6.78	55.74	55.8	43.45	53.49
150	79.97	6.78	62.32	62.65	52.81	61.34
175	87.76	6.78	73.44	73.42	64.67	71.84
200	99.3	6.78	87.07	87.13	74.98	84.39

Table 2: Percentage Scavenging Nitric Oxide radical method

Conc (µg/ml)	Standard	Control	B5	B6	B7	B8
0	0	6.78	0	0	0	0
25	23.08	6.78	15.98	16.72	7.87	6.75
50	34.64	6.78	27.09	28.04	13.21	14.3
75	45.17	6.78	38.23	34.76	16.43	19.94
100	59.29	6.78	49.76	48.76	23.58	24.76
125	67.43	6.78	59.05	60.54	34.16	35.64
150	79.97	6.78	68.67	68.54	42.98	42.78
175	87.76	6.78	76.96	76.51	55.67	56.08
200	99.3	6.78	86.54	85.68	59.06	61.89

Table 3: Percentage Scavenging Superoxide radical method

Conc (µg/ml)	Standard	Control	B1	B2	B3	B4
0	0	7.93	0	0	0	0
25	25.65	7.93	12.76	11.97	10.45	11.67
50	36.64	7.93	21.58	21.54	16.73	19.54
75	45.17	7.93	29.87	28.95	24.24	30.18
100	59.29	7.93	32.56	38.36	32.36	42.56
125	67.43	7.93	56.05	52.81	43.45	56.21
150	79.97	7.93	62.76	62.65	52.81	67.54
175	87.76	7.93	73.41	73.46	64.67	74.76
200	99.3	7.93	86.34	88.21	74.63	85.98

Table 4: Percentage Scavenging
Superoxide radical method

Conc (µg/ml)	Standard	Control	B5	B6	B7	B8
0	0	7.93	0	0	0	0
25	25.65	7.93	16.78	17.45	6.75	7.84
50	36.64	7.93	28.04	29.65	14.3	13.95
75	45.17	7.93	35.78	39.5	19.94	15.78
100	59.29	7.93	48.33	50.32	24.81	22.45
125	67.43	7.93	58.76	60.54	35.64	34.51
150	79.97	7.93	68.61	71.02	42.53	42.79
175	87.76	7.93	77.23	79.87	51.92	55.67
200	99.3	7.93	85.06	87.96	59.54	60.98

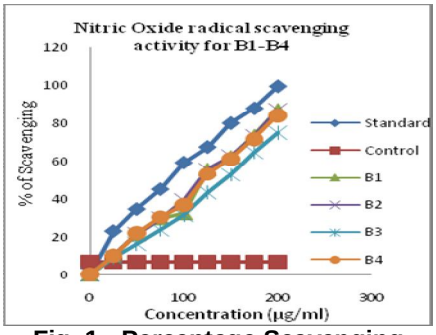


Fig. 1: Percentage Scavenging
Nitric Oxide radical method

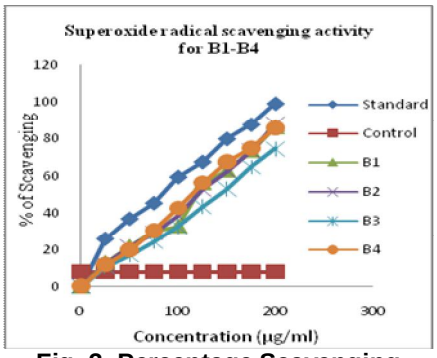


Fig. 3: Percentage Scavenging
Superoxide radical method

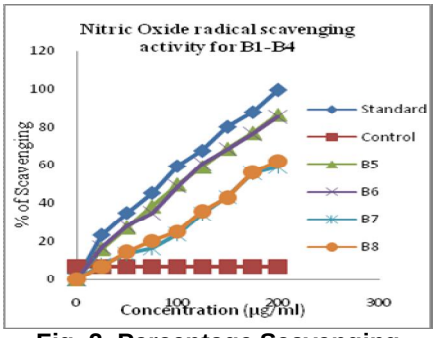


Fig. 2: Percentage Scavenging
Nitric Oxide radical method

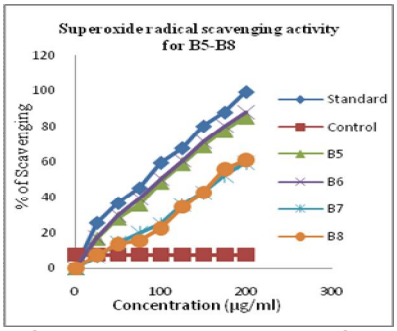


Fig. 4: Percentage Scavenging
Superoxide radical method

Table 5: Anti bacterial activity by cup-borer method

compound code	Anti bacterial activity (Zone of inhibition in mm)							
	B.subtilis		B.pumilis		E.coli		P.Vulgaris	
	50(µg/mL)	100(µg/mL)	50(µg/mL)	100(µg/mL)	50(µg/mL)	100(µg/mL)	50(µg/mL)	100(µg/mL)
B1	10	13	12	16	08	10	09	12
B2	11	16	16	25	11	14	14	19
B3	10	14	12	16	10	13	14	18
B4	14	21	16	25	10	15	11	15
B5	14	18	11	20	12	16	12	18
B6	15	17	12	20	13	15	12	16
B7	13	17	15	19	14	19	12	18
B8	12	16	14	18	12	16	14	20
Ciprofloxacin	28		32		24		30	
Control(DMSO)	-		-		-		-	

Table 6: Anti fungal activity by cup-borer method

	Zone of inhibition (mm)	
compound code	A. niger	C. albicans
	100(µg/mL)	100(µg/mL)
B1	18	14
B2	18	13
B3	17	14
B4	16	14
B5	13	-
B6	14	-
B7	18	11
B8	16	-
Micanazole nitrate	30	27
Control (DMSO)	-	-

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