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Der Pharma Chemica, 2012, 4 (1):141-146
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Antimicrobial activities of synthesized and characterized 5-acetyl pyrimidine-2, 4, 6-(1*H*, 3*H*, 5*H*)-trione based chalcones

Bhavesh D. Dhorajiya, Mahesh H. Malani, Jigar R. Patel and Bharat Z. Dholakiya*

Department of Chemistry, S.V.National Institute of Technology, Ichchanath, Surat, Gujarat (India)

ABSTRACT

A series of eleven 5-((E)-3-phenylacryloyl) pyrimidine-2, 4, 6-(1H, 3H, 5H)-triones (5-acetyl pyrimidine 2, 4, 6-(1H, 3H, 5H) trione based chalcone) were synthesized using diethylmalonate, urea, acetic anhydride and various aryl aldehydes under basic conditions afforded the 5-acetyl pyrimidine-2, 4, 6-(1H, 3H, 5H)-trione based chalcone by means of Claisen–Schmidt condensation reaction. Furthermore, there has been some additional work investigating the effect of these derivatives on biological activity. They were characterized using FT-IR, ^{1}H NMR spectroscopy and elemental analysis. The compounds show anti-fungal & antibacterial activity.

Keywords: 5-acetyl pyrimidine-2, 4, 6-(*1H, 3H, 5H*)-trione based chalcone, Antibacterial activity, antifungal activity.

INTRODUCTION

The medicinal importance of pyrimidine derivatives is significant among various heterocyclic compounds, as they are found to possess anti-neoplastic, antiviral, antibiotic and anti-inflammatory including other biological activities [1]. The pyrimidine ring system being present in nucleic acids, several vitamins, coenzymes, uric acid, purines and some marine microorganisms (e.g. *Sponge*). Many synthetic members of pyrimidine are also important as synthetic drugs (e.g. *Barbituric acid derivatives)* and chemotherapeutic agents (e.g. *Sulfadiazine).* Moreover, these interesting biological activities attracted our attention to the chemistry of nitrogen heterocycles [2]. Many new drugs are envisioned using the small 5-acetyl pyrimidine-2, 4, 6-(1H, 3H, 5H)-trione moiety as a primary building block in the preparation. Direct pharmaceutical and other industrial applications of 5-acylbarbiturates are well-known. [4] Hence, it was considered of interest to determine whether the compounds resulting from the condensation of the 5-acetyl barbituric acid with various aldehyde in presence of base give rise to various chalcone molecules (Scheme 1) would possess significant biological potency such as Antioxidants, Anti-inflammatory, Cytotoxic, Hyperglycemic, Antihepatotoxic, Anti-microbial, Antimalerial, Antileishmanial and Tyrisinase Inhibitors [5], Antiallergic [6], Antiviral [7], Anti HIV [8], Carboxygenase inhibitor [9], Antileishmanial [10], Insecticidal [11-12], Antiulcer [13], Bactericidal [14-15], Fungicidal [16-17] and Anthelmintics [18]. The constitutions of all the

products were characterized using elemental analyses, and IR, ^{1}H NMR and mass spectroscopy. All the compounds were screened for their *in vitro* antimicrobial activity against different strains of bacteria and fungi.

MATERIALS AND METHODS

2.1 Experimental procedure

All reagents were of analytical reagent grade and were used without further purification. Solvents employed were purified by standard procedure before to use. Diethyl malonate and Urea was purchased from Sisco Research laboratory. The melting points were determined in open capillary on Veego (Model: VMP-D) electronic apparatus and are uncorrected. To monitor the reactions, as well as, to establish the identity and purity of reactants and products, thin layer chromatography was performed on microscopic glass slides (2 7.5 cm) coated with silica gel-G, using ethyl acetate-hexane and chloroform–methanol, as the solvent systems, and spots were visualized under UV radiation. Elemental analysis was performed using a Perkin Elmer, USA 2400-II CHN analyser. FTIR spectra (4000–400 cm^{-1}) recorded on Simadzu 8400-S spectrophotometer using KBr disk. Nuclear magnetic resonance spectra were recorded on Varian 400 MHz model spectrometer using DMSO as a solvent and TMS as internal reference (Chemical shifts in δ ppm).

2.2 Synthesis of pyrimidine 2, 4, 6-(1H, 3H, 5H) trione (3)

A solution of diethylmalonate (20 g, 0.5 mol), urea (7.5 g, 0.5), anhydrous sodium methoxide (2.875 g, 0.5 g atom in 62.5 ml in anhydrous methanol) and 62.5 ml methanol was refluxed at 65 ˚C during 7h in a flat bottom flask. A white solid separates rapidly. After in the reaction mixture 125 ml. of hot (50°C) water is added and then enough hydrochloric acid to make the solution acidic. The resulting clear solution is filtered and cooled in an ice bath overnight. The white product was formed from the clear solution. The white product filtered and washed with 50 ml of cold water and then dried in an oven at 105–110 °C for 3-4h to afford the title compound (10.4 g, 65 %), as a white powder, m.p. 252-257 ˚C (Decomposed) [19].

2.3 Synthesis of to 5-acetyl pyrimidine 2, 4, 6-(1H, 3H, 5H) trione (4)

A solution of pyrimidine 2, 4, 6-(1H, 3H, 5H) trione (6.4 g, 0.05 mol), acetic anhydride (150 ml) and few drops of H_2SO_4 acid was refluxed for 1 h. The reaction in the beginning was a suspension but after about 10 min of refluxing it changes color (orange) and becomes a solution. The reaction mixture was concentrated in to 1/2 of its original volume and cooled at about 10°C. The solid product was formed, filtered, washed with hot water then acetone and dried at 80°C for 30 min to give compound (7.8 g, 92%) yield, as a yellow powder, m.p. 242-246 (Decomposed) [5].

Table 1.List of compounds with their different functional groups

Compounds	R1, R2, R3 Groups		
5a	H	H	H
5b	OH	H	H
5c	H	H	OH
5d	H	H	OMe
5e	H	OMe	OMe
5f	Cl	H	H
5g	H	H	Cl
5h	H	NO_2	H
5i	H	H	NO_2
5j	H	H	Me
5k	H	H	1,3-Butadiene in ring

2.4 General procedure for Synthesis of chalcones (5a–k).

The target compounds were prepared as shown in Scheme 1. Claisen–Schmidt condensation of 5-acetyl pyrimidine-2, 4, 6-(1H, 3H, 5H)-trione and various aryl aldehydes under basic condition afforded pyrimidine based chalcone. [5, 19] New compounds were completely characterized based on their spectroscopy data. Derivatives are shown in Table. 1

2.5 Antibacterial and anti-fungal studies

All the synthesized compounds were tested for their antibacterial activity (MIC) in vitro by the karbi broth dilution method with bacterial strain such as Pseudomonas aeruginosa, Escheria Coli, Staphycocus and Basillus Subtilis taking Standard Drugs (Ciprofloxacin) and Fungal Strain Candida Albicans (MIC) taking Standard Drugs (Flucanazole) respectively.[21]

RESULTS AND DISCUSSION

Physiochemical parameters of the compounds are presented in Table 2. All the compounds were colored and stable in air. They were insoluble in water but soluble in organic solvents like CHCL3, DMF and DMSO.

3.1 IR & NMR Spectral Studies

IR Spectra of compound

The important infrared spectral bands and their tentative assignments for 5-acetyl pyrimidine-2, 4, 6-(1H, 3H, 5H)-trione and its chalcones were recorded as KBr disks and are presented. The IR spectra of compounds 5a–h revealed a characteristic bands between 710 cm^{-1} and 703 cm^{-1} confirming the presence of (C=C) groups. IR spectrum of the compounds showed a characteristic bands between 1694 cm^{-1} and 1716 cm^{-1} confirming the presence of C=O groups and C–Cl present between 764 cm^{-1} and 766 cm^{-1} of aromatic ring. IR spectrum of the compound 5c showed a characteristic band at 1351 cm^{-1} and 1355 cm^{-1} confirming the presence of $–NO_2$ group. [21]

Table 2.Physicochemical parameter of the compounds

				Analytical (%) Found (Calculated)				
Sr. No	Formula Weight	Color (Yield %)	Melting Point (˚C)	C	H	N	O	Cl
3	134.13	White (65)	252-257	35.82 (35.79)	7.51 (7.53)	20.88 (20.91)	35.78 (35.76)	
4	176.17	Yellow (92)	242-246	40.91	6.87	20.88	36.33	
5a	258.23	Yellow(89)	184-188	60.47 (60.45)	3.09 (3.05)	10.85 (10.87)	24.78 (24.76)	-
5b	274.22	Brown (81)	293-296	56.94 (56.91)	3.68 (3.65)	10.22 (10.25)	29.17 (10.21)	-
5c	274.22	Yellow (79)	206-210	56.94 (56.97)	3.68 (3.72)	10.22 (10.24)	29.17 (29.14)	-
5d	288.26	Yellow (83)	228-233	58.33 (58.31)	4.20 (4.23)	9.72 (9.75)	27.75 (27.77)	-
5e	318.28	Yellow(73)	191-196	56.60 (56.58)	4.43 (4.26)	8.80 (8.82)	30.16 (30.18)	-
5f	292.67	Yellow(69)	237-242	53.35 (53.38)	3.10 (3.08)	9.57 (9.55)	21.87 (21.89)	12.11 (12.14)
5g	292.67	Yellow(80)	258-263	53.35 (53.33)	3.10 (3.13)	9.57 (9.58)	21.87 (21.84)	12.11 (12.13)
5h	303.23	Yellow(78)	203-208	51.49 (51.46)	2.99 (2.97)	13.86 (13.89)	31.86 (31.91)	-
5i	303.23	Orange(71)	209-213	51.49 (51.48)	2.99 (2.96)	13.86 (13.87)	31.86 (31.84)	-
5j	272.256	Yellow(87)	182-187	61.76 (61.74)	4.44 (4.46)	10.29 (10.31)	23.51 (23.49)	-
5k	308.29	Yellow(93)	187-193	66.23 (66.25)	3.92 (3.95)	9.09 (9.11)	20.76 (20.71)	-

The ^1H NMR spectra revealed signals 3.50 δ ppm appears as signals for DMSO solvent. The important infrared spectral bands and their tentative assignments for 5-acetyl pyrimidine-2, 4, 6-(1H, 3H, 5H)-trione and its chalcones were recorded as KBr disks and are presented. The ^1H NMR spectra revealed signals 3.50 δ ppm appears as signals for DMSO solvent. The ^1H NMR data of compounds revealed signals between 4.19 and 4.25 δ ppm for CH of pyrimidine ring. The ^1H NMR data of compounds revealed signals between 6.52 and 6.92 δ ppm for OH of pyrimidine ring. The ^1H NMR data of compounds revealed signals between 10.61 and 11.84 δ ppm NH of

pyrimidine ring. The ^{1}HNMR data of compounds revealed signals between 7.01 and 8.14 δ ppm for aromatic protons of substituted phenyl ring. [22]

Table 3. The characteristic IR bands of the compounds

Sr. No	ν (-NH) cm^{-1}	ν (C=O) cm^{-1}	ν (C=C) cm^{-1} (Aromatic)	ν (C-NO_2) cm^{-1}	ν (O-C) cm^{-1}	ν (-C-Cl) cm^{-1}
3	3478	1716(s)	-	-	1138(m)	-
4	3440	1694(s)	-	-	1116(m)	-
5a	3438	1696(s)	1612(s)		1136(m)	-
5b	3436	1698(s)	1614(s)	-	1138(m)	-
5c	3437	1697(s)	1616(s)	-	1140(m)	-
5d	3439	1698(s)	1613(s)	-	1136(m)	-
5e	3436	1699(s)	1615(s)	-	1138(m)	-
5f	3434	1701(s)	1620(s)	-	1137(m)	764
5g	3438	1700(s)	1621(s)	-	1139(m)	766
5h	3436	1700(s)	1617(s)	1351 (s)	1139(m)	-
5i	3437	1698(s)	1618(s)	1355(s)	1142(m)	-
5j	3443	1695(s)	1614(s)	-	1137(m)	-
5k	3439	1698(s)	1619(s)	-	1141(m)	-

Table 4. Chemical shifts values of the ^{1}H NMR spectra of the compounds

Compounds code No.	Chemical shifts of the ^{1}H NMR spectra of the compounds from 3 to 5k
3	2.94 δ ppm (2H,s,-CH2), 3.50 δ ppm (CH3 of DMSO solvent),5.90 δ ppm (1H,s, CH pyrimidine), 6.73 δ ppm (3H,m, OH of pyrimidine ring), 11.63 δ ppm (1H, s, NH),11.84 δ ppm (1H, s, NH).
4	2.64 δ ppm (3H,s,-CH3), 3.50 δ ppm (CH3 of DMSO solvent), 7.73 δ ppm (1H,s, cyclic ring at C5), 10.63 δ ppm (1H, s, NH),11.84 δ ppm (1H, s, NH).
5a	2.60 δ ppm (1H, s, -CHAr=CH), 2.64 δ ppm(1H,s, -CHCOAr=CH), 3.50 δ ppm (CH3 of DMSO solvent),4.24 δ ppm (1H, s,-CH of pyrimidine ring at C5), 6.58-7.98 δ ppm (5H, m, Ar-H) 10.63 δ ppm, (1H, s, NH), 10.84 δ ppm (1H, s, NH).
5b	2.58 δ ppm (1H, s, -CHAr=CH), 2.65 δ ppm(1H,s, -CHCOAr=CH), 3.50 δ ppm 4.22 δ ppm (1H, s,-CH of pyrimidine ring at C5), 6.52-6.89 δ ppm (1H, s, C-OH), 7.76-7.97 δ ppm (4H, m, Ar-H), 10.61 δ ppm, (1H, s, NH), 10.85 δ ppm (1H, s, NH).
5c	2.61 δ ppm (1H, s, -CHAr=CH), 2.66 δ ppm(1H,s, -CHCOAr=CH),3.50 δ ppm 4.19 δ ppm (1H, s,-CH of pyrimidine ring at C5), 7.43-7.68 δ ppm (4H, m, Ar-H), 6.67-6.77 δ ppm (1H, s, C-OH), 10.55 δ ppm, (1H, s, NH) 10.69 δ ppm (1H, s, NH).
5d	2.62 δ ppm (1H, s, -CHAr=CH), 2.65 δ ppm(1H,s, -CHCOAr=CH), 3.09 δ ppm (3H,s, -OCH3), 4.20 δ ppm (1H, s,-CH of pyrimidine ring at C5), 6.67-7.79 δ ppm (4H, m, Ar-H), 10.79 δ ppm, (1H, s, NH) 10.62 δ ppm (1H, s, NH).
5e	2.57 δ ppm (1H, s, -CHAr=CH),2.63 δ ppm(1H,s, -CHCOAr=CH), 2.98 δ ppm (6H,s, 2-OCH3), 4.24 δ ppm (1H, s,-CH of pyrimidine ring at C5), 6.67-7.75 δ ppm (3H, m, Ar-H), 10.68 δ ppm, (1H, s, NH) 10.89 δ ppm (1H, s, NH).
5f	2.59 δ ppm (1H, s, -CHAr=CH), 2.66 δ ppm(1H,s, -CHCOAr=CH), 4.25 δ ppm (1H, s,-CH of pyrimidine ring at C5), 6.59-7.94 δ ppm (4H, m, Ar-H), 10.62 δ ppm, (1H, s, NH) 10.81 δ ppm (1H, s, NH).
5g	2.56 δ ppm (1H, s, -CHAr=CH), 2.63 δ ppm(1H,s, -CHCOAr=CH), 4.23 δ ppm (1H, s,-CH of pyrimidine ring at C5), 6.71-7.24 δ ppm (4H, m, Ar-H), 10.65 δ ppm, (1H, s, NH) 10.77 δ ppm (1H, s, NH).
5h	2.57 δ ppm (1H, s, -CHAr=CH), 2.67 δ ppm(1H,s, -CHCOAr=CH), 4.24 δ ppm (1H, s,-CH of pyrimidine ring at C5), 6.92-8.07 δ ppm (4H, m, Ar-H), 10.68 δ ppm, (1H, s, NH) 10.75 δ ppm (1H, s, NH).
5i	2.59 δ ppm (1H, s, -CHAr=CH), 2.61 δ ppm(1H,s, -CHCOAr=CH), 4.27 δ ppm (1H, s,-CH of pyrimidine ring at C5), 6.76-8.14 δ ppm (4H, m, Ar-H), 10.66 δ ppm, (1H, s, NH) 10.72 δ ppm (1H, s, NH).
5j	2.61 δ ppm(1H,s, -CHCOAr=CH) 2.85 δ ppm (3H, s, CH3), 4.22 δ ppm (1H, s,-CH of pyrimidine ring at C5), 6.75-7.89 δ ppm (4H, m, Ar-H), 10.69 δ ppm, (1H, s, NH) 10.78 δ ppm (1H, s, NH).
5k	2.59 δ ppm (1H, s, -CHAr=CH), 2.67 δ ppm(1H,s, -CHCOAr=CH), 4.24 δ ppm (1H, s,-CH of pyrimidine ring at C5), 6.68-7.71 δ ppm (7H, m, Ar-H), 10.85 δ ppm, (1H, s, NH) 10.62 δ ppm (1H, s, NH).

3.2 Antibacterial Activity

The antibacterial activity of compounds was studies with four pathogenic bacteria (Table 5). Ciprofloxacin were used as reference for inhibitory activity against bacteria. Most of the synthesized compounds showed negligible antibacterial activity. When compared with the compounds 5d, 5i, 5j, the activity was comparable with Ciprofloxacine in the case of Pseudomonas aeruginosa, as well as 5d and 5i in the case of Bacillus Subtilis.

3.3 Antifungal Activity

The antifungal activity of all compounds was studies with one pathogenic fungus (Table 6). Flucanazole were used as reference for inhibitory activity against fungi. All the synthesized compounds showed negligible activity. None of the above compounds are potent active towards pathogenic fungi.

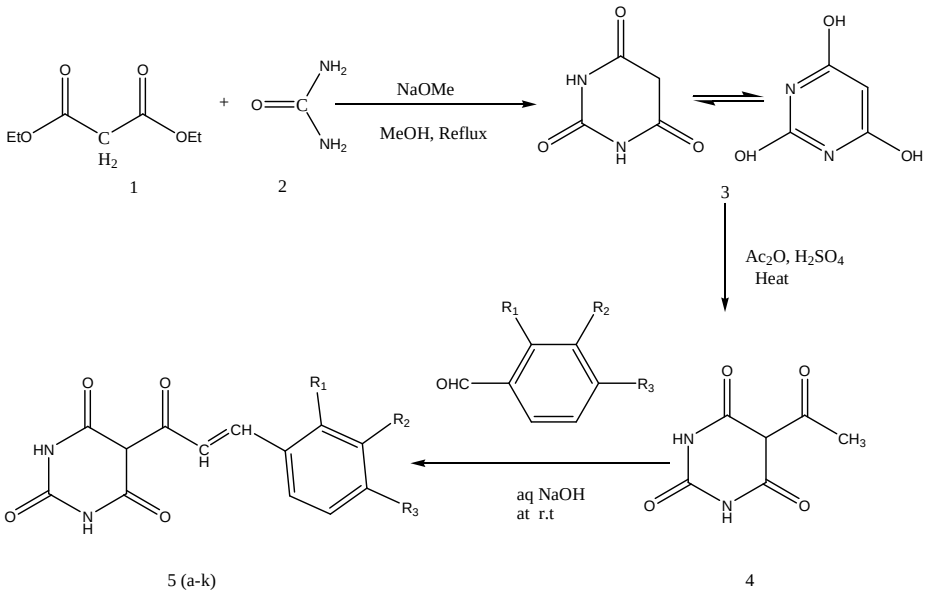
Table 5. Anti-bacterial Activity of the compounds

Compounds Code No.	Minimal Inhibitory Concentration (MIC) (µg/ml)			
	Bacterial Species			
	Gram Negative Bacteria		Gram Positive Bacteria	
	Escheria Coli	*Pseudomonas aeruginosa*	*Staphycocus*	*Basillus Subtilis*
5a	300	>600	>600	600
5b	600	600	>600	100
5c	600	600	>600	600
5d	20	>600	300	20
5e	300	>600	>600	600
5f	300	>600	>600	>600
5g	300	>600	>600	>600
5h	300	600	600	>600
5i	20	600	600	60
5j	20	>600	>600	100
5k	300	>600	>600	>600
Standard Drugs (Ciprofloxacin)	10	20	10	05

Table 6. The antifungal activity of the compounds

Compounds Code No	Minimal Inhibitory Concentration (MIC) (µg/ml)
	Fungal Species Candida Albicans (MIC)
5a	300
5b	600
5c	300
5d	600
5e	600
5f	600
5g	600
5h	600
5i	300
5j	300
5k	>600
Standard Drugs (Flucanazole)	10

Scheme 1



CONCLUSION

The present investigation revealed 5-acetyl pyrimidine-2, 4, 6-(1H, 3H, 5H)-trione based chalcones as potential leads for development of new drugs. We can also conclude from the result

of antibacterial activity of compounds 5d, 5i and 5j comparable with the standard drugs Ciprofloxacin in the case of Pseudomonas aeruginosa. Comparative analysis showed Moderate microbial activity of all the compounds compared with the standard drug Ciprofloxacin. In future 1-(2, 4, 6-trihydroxypyimidine-5-yl) ethanone derivatives will be used for further development of new cytotoxic agent.

Acknowledgment
The authors wish thank to Department of Applied Chemistry, SVNIT, Surat for providing laboratory facilities & financial support, Paresh Kapopara, Central Diagomnistic Laboratory, Surat, for antimicrobial activity determinations. Analytical facilities providing by the Centre of Excellence, Vapi and SAIF, Punjab University, Chandigarh, India are gratefully acknowledged.

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