Available online at www.worldscientificnews.com

World Scientific News

An International Scientific Journal

WSN 117 (2019) 212-220 EISSN 2392-2192

SHORT COMMUNICATION

Synthesis characterization and antimicrobial activity of ethyl-4-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxylate

M. Pasupathi[1,a], N. Santhi[1,b]

[1]PG & Research Department of Chemistry, Government Arts College, C. Mutlur, Chidambaram, Tamil Nadu, India

[2]Government Arts College, C. Mutlur, Chidambaram, Tamil Nadu, India

[a,b]E-mail address: pasumanichem@gmail.com , nsanthi@gmail.com

[a]Mobile: +91 9976595699

[b]Mobile: +91 9843773445

ABSTRACT

Heteroatoms like nitrogen, sulfur, and oxygen containing biologically active ethyl 4-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxylate was synthesized from 2,4-dichloro benzaldehyde, acetoacetic ester and thiourea using AlTiTUD-1 as a catalyst. The synthesized compound was characterized by FT-IR, ^{1}H and ^{13}C NMR spectral values. The compound possesses good antimicrobial activity is determined by well diffusion method against gram positive, gram negative and fungal species. The minimum inhibitory concentration value of the compound was determined by broth dilution method.

***Keywords*:** pyrimidine, antimicrobial, MIC, mesoporous

(Received 26 December 2018; Accepted 05 January 2019; Date of Publication 06 January 2019)

1. INTRODUCTION

Pyrimidine and their derivatives are having vital role in the field of drugs and agricultural chemicals. Pyrimidine could be a basic nucleus (uracil, thiamine and cytosine) of the six nucleus found in DNA & RNA[1]. It is also possesses wide range biological activities[2]. The living organism contains pyrimidine as one of the most important members of all the diazines in the ring system. Many pyrimidine derivatives are widely distributed in nature, and possesses wide range of pharmacological properties such as antitumor[3-6], anticancer[7], antiviral[8], antifungal, antibcterial[9], antimicrobial[10], antiinflammator[11-14], analgesic[15], anti-HIV[16], antiplatelet[17], antithrombotic[17], activities, etc. The discovery of antibiotics was very important tool in the early twentieth century to treat bacterial diseases; the resistance to human pathogens increasing against the current antimicrobial agents creates a severe problem in the field of medicinal chemistry. In addition, sometimes antibiotics are causes adverse effects on the host including the problems like hypersensitivity, immune suppression and allergic reactions. Hence it is important to search and synthesis of a new class of antimicrobial compounds which are effective against pathogenic microorganisms developing resistance to the antibiotics and with lesser side effects to the host[1]. There are a number of different classes of antibacterial and antifungal agents have been discovered[18-19].

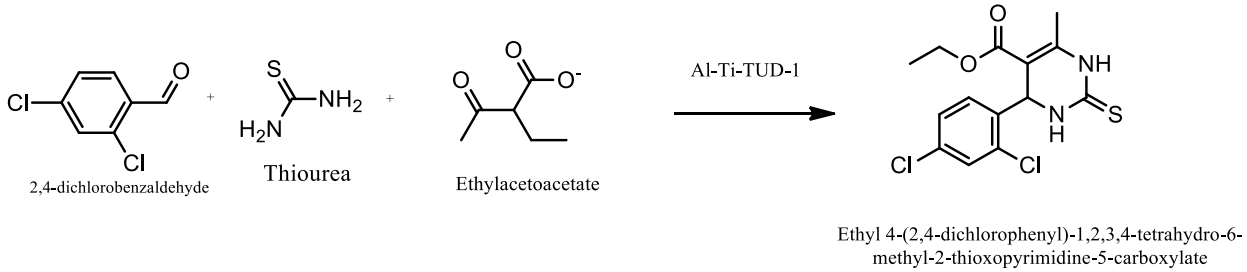
There has been much progress in this field of drug development after the discovery of several synthetic and semi-synthetic antibacterial sulfa drugs, penicillins nitrofuranes, cephalosporins, tetracyclines, macrolides, oxazolidinones and antifungal agents such as, ketoconazole, miconazole, including amphotericin B and fluconazole. Still many problems are there to be solved for most antimicrobial drugs available despite advances in antibacterial and antifungal therapies. Nowadays multi-drug resistant is developing due to the extensive use of antibiotics against the microbial pathogens[20]. In this regard the synthesis of pyrimidine derivatives have been an attracting extensive attention, as a wide range of such compounds played an important role in the field of medicinal chemistry as analgesic[21-24] anti-inflammatory[25-26], antimicrobial[27-29], anticancer activities[30-34] etc. A large number of review has been reported in the synthesis of substituted Pyrimidine and their biological importance[35,36].

The most common and broadly working route to pyrimidine synthesis is the combination of a reagent containing the N-C-N skeleton with C-C-C unit. In N-C-N reagent both the nitrogen atoms are act as nucleophiles and in C-C-C reagents the terminal carbon atoms of are acts as electrophiles. Generally urea, thiourea and guanidine are used as N-C-N reagents and 1,3-diketones, diesters and dinitriles are used as C-C-C skeleton containing reagents. When the ethoxy carbonyl group is substituted in position 5 of the pyrimidine ring is, that it is more susceptible to form modified bases of significant structural importance. Thus, the present work report on the synthesis of ethyl 4-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxylate. The structure of the compound was characterized by FT-IR, ^{1}H and ^{13}C NMR spectral values.

2. MATERIALS AND METHODS

2,4-dichlorobenzaldehye, thiourea, ethylacetoacetate were purchased from sigma aldrich and the remaining chemicals were purchased from either sigma Aldrich or Merck and are analytical grade. Melting points were determined by the open tube capillary and are

uncorrected. The purity of the compound was checked by thin layer chromatography (TLC) plates (silica gel G) in the solvent system toluene:ethtyl acetate (7.5:2.5). The spots were identified by exposure to iodine vapors. The IR spectrum was recorded in Perkin-Elmer 1720 FT-IR spectrometer (KBr pellets). The ^{1}H and ^{13}C NMR spectra were obtained by Bruker Advance 500 MHz spectrometer using TMS as the internal standard in DMSO. The compound was synthesized by the method previously reported in our paper[20].



Scheme - 1

Characterization of ethyl-4-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxylate

Yield 82%, mp. 281-283 ºC, IR (KBr) in cm^{-1}: 3400, 3195 (-NH, st.), 1707 (-C=O, st.), 2977 (-CH, aliphatic), 3095 (-CH, phenyl ring), 1570 (-C=S, st.), 1380 (-C-N-C- in pyrimidine ring), 1461 (-C-N, st); ^{1}H NMR (DMSO, 500 MHz, in ppm), δ 1.06 (t, 2H, CH_3 of CH_2CH_3), 2.30 (s, 3H, CH_3), 3.92 (q, 2H, CH_2 of CH_2CH_3), 5.60 (s, 1H, -CH of pyrimidine ring), 7.29-7.73 (m, 3H, benzene), 9.62 (s, 1H, -NH in pyrimidine ring), 10.34 (s, 1H, -NH in pyrimidine ring); ^{13}C NMR (DMSO, 125 MHz); δ 14.34, 17.51, 51.72, 59.99, 99.78, 128.52-129.45, 140.29, 146.34, 165.10, 174.30.

2. 1. Microbial activity - procedure

2. 1. 1. Preparation of Inoculums

Stock cultures were maintained at 4 ºC on slopes of nutrient agar. To prepare the active cultures of experiment, a loopful of cells were transferred from the stock cultures to test tube of Muller-Hinton broth (MHB) for the bacteria incubated without agitation for 24 hrs at 37 ºC. The cultures were adulterated with fresh Muller-Hinton broth to obtain optical densities equivalent to 2.0 X 106 colony forming units (CFU/ml) for bacteria.

2. 1. 2. Antibacterial Assay

The antimicrobial activity was screened by using well diffusion method. In vitro antimicrobial activity was determined by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The petri plates were sterile and 15 ml of molten media was poured into that petri plates to prepare Muller Hinton Agar plates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. Wells were cut and 20 µl of the different concentration of ethyl-4-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxylate in ethanol was added. The plates were then incubated at 37 °C for 24 hours. The antibacterial

activity was determined by measuring the diameter of the inhibition zone (in mm) formed around the well. Chloramphenicol disc was used as a positive control.

Table 1. Antibacterial Activity of ethyl-4-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxylate.

S. No	Microorganisms	Zone of inhibition (in mm)				
		100	75	50	25	+ve Control
Gram Positive						
1.	*Rhodo coccus*	18	16	13	11	26
2.	*S. aureus*	19	17	15	13	27
3.	*B. subtilis*	17	14	11	09	24
4.	*Enterococcus faecalis*	16	15	12	10	22
Gram Negative						
5.	*E. coli*	19	18	16	14	28
6.	*Klebsiella pneumonia*	16	13	12	10	23
7.	*S. typhi*	17	15	13	11	25
8.	*Serratia mercescens*	18	16	15	13	27

2. 1. 3. Antifungal Assay

To determine the antifungal activity totally two fungal strains were used throughout investigation. All the fungal cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young fungal broth cultures were prepared before the screening procedure.

2. 1. 4. Antifungal activity

Antifungal activity was also measured using methods of well diffusion plates on agar. In order to test the antifungal activity, the fractions of different concentration of ethyl-4-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxylate was dissolved in 70% ethanol. About 20 ml of Sabouraud Dextrose Agar was poured into each 15 cm Petri dish. C. albicans and A. niger were grown in Sabouraud Dextrose Broth at 27 ºC for 48 hrs. Growth was adjusted to OD (600 nm) of 0.1 by dilution with Sabouraud Dextrose Broth . Then, Wells were cut and 20 µl of the different concentration ethyl-4-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxylate in ethanol was located on agar to load 10 and 15 µl of each spice sample (1 mg/ml). 100 units of Fluconazole, was used as a positive

control and was obtained from a local pharmacy. Inhibition zones were measured (in mm) after incubation at 27 ºC for 48 hrs.

Table 2. Antifungal Activity of ethyl-4-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxylate.

S. No	Microorganisms	Zone of inhibition (in mm)				
		100	75	50	25	+ve Control
1.	*Aspergillus nigar*	15	13	12	10	23
2.	*Candida albicans*	16	14	11	09	25

2. 2. Minimum Inhibitory concentration

The Minimum Inhibitory Concentration Assay is a useful technique to determine the lowest concentration of a particular antibiotic needed to kill bacteria. This assay is typically performed on planktonic (free floating) bacterial cells. MIC is in general helpful in establishing the level of resistance of a specific bacterial strain and can extensively affect the decision to use certain antimicrobial agents. Broth dilution method was used to prepare the test solution which allows the choice of providing both quantitative (MIC) and qualitative (category interpretation) results.

The MIC of a chemical is determined by preparing solutions of the chemical in vitro at increasing concentrations, incubating the solutions with the separate batches of cultured bacteria, and the results are measured by using broth dilution method. Results obtained have been graded based on the intensity of the turbidity formed in the tubes into – (no growth), * (considerably arrest), + (cloudy solution), ++ (Turbid solution), +++ (Highly turbid solution), (-) indicates no zone of inhibition formed.

During testing, multiple microtiter plates are filled with a broth composed of Brucella and supplements of blood[37]. Different concentrations of the synthesized compound and the bacteria to be tested are then added to the plate. Then the plate is placed into a non-CO_2 incubator fifteen to twenty hours at the temperature of 35 ºC. After the sufficient time, the plate is removed and tested for bacterial growth. If the broth developed cloudy or a layer of cells formed at the lowermost part of the tube, then it shows bacterial growth has occurred. The results of the broth dilution method are reported in Minimum Inhibitory Concentration (MIC), or the lowest concentration of antibiotics that stopped bacterial expansion in Table 3.

The amount of microbe growth is determined by the turbidity formed in the tubes. The tube with least turbid or clear tubes is correlated with the absence of microbes. The tube with no antimicrobial agent presents as cloudy and most turbid because the microbes are able to flourish in the absence of antibiotic. As antimicrobial concentration increases, the turbidity decreases until the MIC is reached and microbes can no longer survive. Antimicrobials with low MICs are more effective than those with High MICs, as only a low dosage is needed to exterminate microbes.

3. RESULT AND DISCUSSION

Antibacterial effects of the ethyl-4-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxylate against totally eight bacterial strains and two fungal strains were used throughout investigation. The bacteria used were *Rhodo coccus, Staphylococcus aureus, Bacillus subtillis, Enterococccus faecalis, E.coli, Klebsiella pneumonia, S. typhi* and *Serratia mercescens.* The fungal strains used were *Aspergillus niger* and *Candida albicans.* The antibacterial activity of the tested of compound name showed significant reduction in bacterial growth in terms of zone of inhibition. The compound showed dose dependent activity i.e., while increase in the concentration of extract, the zone of inhibition is also increased.

Table 3. Minimum Inhibitory concentration assay of ethyl-4-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxylate.

S. No	Microorganisms	Turbidity of the microbial tubes				
	Volume of microbial rich broth	**100**	**75**	**50**	**25**	**12.5**
	Volume of antimicrobial agent	**12.5**	**25**	**50**	**75**	**100**
Gram Positive						
1.	Rhodo coccus	+ + +	+ + +	+ + +	+ +	+ +
2.	S.aureus	+ + +	+ + +	+ + +	+ + +	+ +
3.	B.subtilis	+ + +	+ + +	+ + +	+ +	+ +
4.	Enterococcus faecalis	+ + +	+ + +	+	+	+
Gram Negative						
5.	E.coli	+ +	+ +	+ +	+	+
6.	Klebsiella pneumonia	+ + +	+ + +	+ +	+ +	+ +
7.	S.typhi	+ + +	+ `	+ + +	+	+
8.	Serratia mercescens	+ + +	+ +	+ +	+ + +	+ +
Fungal						
9.	Aspergillus niger	+ + +	+ + +	+ + +	+ +	+ +
10.	Candida albicans	+ + +	+ + +	+ + +	+ +	+ +

MIC Concentration; - No growth; * - considerably arrest; + cloudy solution; ++ - Turbid solution; +++ - Highly turbid solution; (-) indicates no zone of inhibition formed.

From Table 1 it is known that ethyl-4-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxylate has good antibacterial activity against both gram positive and gram negative bacteria but lesser than that of positive control Chloramphenicol. Similarly in Table 2 ethyl-4-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxylate shows better activity against the fungul stain also. The positive control used against the fungal is 100 units of Fluconazole. It is observed that the activity of the compound against microorganisms increased on increasing the concentration of the ethyl-4-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxylate compound. The activity of the compound is more or less equal against all the microorganisms at all the concentrations is observed from the Table 1 and 2. Hence the minimum inhibitory concentration was done for the same microorganisms.

From Table 3 it is observed that in average the compound ethyl-4-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxylate having effective MIC at 75% of concentration. More over the compound is having 50% of MIC against the gram positive bacteria *Enterococcus faecalis.* At the same time it has the poor MIC values against the gram positive bacteria *S. aureus* and gram negative bacteria *Serratia mercescens.* In both the bacteria the compound shows highly turbid solution upto 75% of antimicrobial concentration. The compound have moderate activity against remaining all microorganisms except the bacteria discussed above.

4. CONCLUSIONS

We have synthesized ethyl-4-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxylate using newly synthesized mesoporous acidic catalyst Al – Ti - TUD-1. The biological evaluation of the synthesized compound was carried out against four gram positive, four gram negative and two fungal microorganisms. The compound has shown good activity against all the microorganisms taken for analysis. The MIC value of the compound also found and it has lower MIC against the gram positive bacteria Enterococcus faecalis. Hence from the above results the biological activity of the compound ethyl-4-(2,4-dichlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimidine-5-carboxylate against some microorganisms is explored.

References

[1] M. Koca, S. Servi, C. Kirilmis, M. Ahmedzade, C. Kazaz, B. Ozbek, G. Otuk. *Eur. J. Med. Chem.* 40 (2005) 1351.

[2] K.M. Ghoneim, R. Youssef, *J Indian Chem Soc.* 53 (1986) 914.

[3] N. Zhang, S. Ayral-Kaloustian, T. Nguyen, R. Hernandezb, C. Beyerb, *Chem Lett* 17 (2007) 3003.

[4] N.R. Mohamed, M.M. Talaat El-Saidi, Y.M. Alia, M.H. Elnagdi, *Bioorg Med Chem.* 15 (2007) 6227.

[5] E.M. Rakib, S. Abouricha, A. Hannioui, N. Benchat, L. Ait M'barek, A. Zyad, *J Iranian Chem Soc.* 3 (3) (2006) 272.

[6] P.G. Baraldi, M.G. Pavani, M. Nunez, et al., *Med Chem Lett.* 10 (2002) 449.

[7] A.M. Kamela, B. Munsonb, *J Ame Soc Mass Spectr.* 18 (2007) 1477.

[8] N. Zhang, S. Ayral-Kaloustian, T. Nguyen et al., *J Med Chem.* 50 (2007) 319.

[9] O. Prakash, R. Kumar, R. Kumar, P. Tyagi, R.C. Kuhad, *Euro J Med Chem.* 42 (2007) 868.

[10] S.M. Sondhi, S. Jain, M. Dinodia, R. Shuklab, R. Raghubir, *Bioorg Med Chem.* 15 (2007) 3334.

[11] P. Molina, E. Aller, A. Lorenzo, et al., *J Med Chem.* 44 (2001) 1011.

[12] S.S. Bahekar, D.B. Shinde, *Acta Pharm.* 53 (2003) 223.

[13] S.M. Sondhi, M. Johar, S. Rajvanshi, et al., *Aust J Chem.* 54 (2001) 69.

[14] M.C. Lanier, M. Feher, N.J. Ashweek, et al., *Bioorg Med Chem.* 15 (2007) 5590.

[15] F.A. Eid, A.F.A. El-Wahab, G.A.M. El-Hag Ali, M.M. Khafagy, *Acta Pharm.* 54 (2004) 13.

[16] L. Xiaoling, G. Mei-Lin, *Bioorg Med Chem.* 14 (2006) 153.

[17] O. Bruno, C. Brullo, S. Schenone, et al., *Med Chem.* 14 (2006) 121.

[18] Appelbaum and Hunter, P.C. Appelbaum, P.A. Hunter, *Int. J. Antimicrob. Agents.* 16 (2000) 5.

[19] Andriole, V.T. AndrioleIn, Current Clinical Topics in Infectious Diseases Blackwell Sciences. (1998) p. 19.

[20] Frere, J.M. Frere, *Mol. Microbiol.* 16 (1995) 385.

[21] Sondhi MS, Jain S, Dinodia M, Shukla R, Raghubir R, *Bioorg Med Chem.* 2007, 15(10) 3334-44.

[22] Bruno O, Brullo C, Schenone S, Francesco B, Ranise A, Massimiliano T et al., *Bioorg Med Chem.* 2004, 12(3), 553-561.

[23] Alagarsamy V, Meena S, Ramseshu KV, Solomon VR, Thirumurugan K, Dhanabal K, *Eur J Med Chem.* 41(11) (2006) 1293-1300.

[24] Zhou JP, Ding YW, Zhang HB, Xu L, Dai Y. Chin, *Chem Lett* 2008, 19(6), 669-672.

[25] Keri RS, Hosamani KM, Shingalapur RV, Hugar MH, *Eur J Med Chem.* 2010, 45(6), 2597-605.

[26] Devesa I, Alcaraz JM, Riguera R, Ferrandiz ML, *Eur J Pharmacol* 2004, 488(1-3): 225-230.

[27] Shaaban MR, Saleh TS, Mayhoub AS, Mansour A. Farag AM, *Eur J Med Chem.* 2008, 16(12), 6344-6352.

[28] Antre RV, Kumar AC, Goli D, Andhale GS, Oswal RJ, *Saudi Pharm J* 2011, 19(4), 233-243.

[29] Mohamed MS, Awad SM, Sayed AI. *Molecules.* 2010, 15(3), 1882-1890.

[30] Vaghasia SJ, Shah VH. Microwave assisted synthesis and antimicrobial activity of novel pyrimidine derivatives, *J Serb Chem Soc.* 2007, 72(2), 109-117.

[31] Salahuddin Md, Singh S, Shantakumar SM. *Rasayan J Chem.* 2009, 2(1), 167-73.

[32] Deshmukh MB, Salunkhe SM, Patil DR, Anbhule, *Eur J Med Chem.* 2009, 44(6), 2651-2654.

[33] Bekhit AA, Fahmy HTY, Rostom SAF, Baraka AM, *Eur J Med Chem.* 2002, 38(1), 27-36.

[34] Naik TA, Chikhalia KH, *E J Chem* 2007, 4(1), 60-66.

[35] G.W. Kenner, A. Todd, Heterocyclic Compounds. R.C. Elderfield (Ed.), Vol. 6, Wiley, New York (1957)

[36] D.J. Brown, The Chemistry of Heterocyclic Compounds A. Weissberger (Ed.), vol. 16, Interscience, New York (1962)

[37] Lorian, Victor (2005), Antibiotics in Laboratory Medicine. Lippincott Williams & Wilkins, p. 149.