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Synthesis and In-Vitro Analysis of Azetidinone Derivatives as Anthelmintic and Anti-Bacterial Agents

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ABSTRACT

Herein, a series of azetidinone derivatives incorporating dihydropyrimidinone moiety **4(a-h)** were prepared. The structures of newly synthesized compounds were confirmed on the basis of elemental analysis, FTIR and ^{1}H NMR spectroscopy. The synthesized compounds were evaluated for their anthelmintic and anti-bacterial activity and it was found that the compounds were pharmacological active and gave appreciable results.

Keywords: Azetidinone derivative; anthelmintic; anti-bacterial.

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INTRODUCTION

Helminth infections are among the most widespread infections in humans, affecting a huge population of the world. Infections with helminthes or parasitic worms affect more than two billion people worldwide. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas[1-3]. The worms in general, reside in the gastrointestinal tract but may also burrow into the liver and other organs[4]. They produce harmful effect on host by depriving him of food, causing blood loss and by secreting toxins[5,6]. These gastrointestinal helminthes become resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases[7]. Anthelmintic are drugs that act locally to expel parasitic worm from gastrointestinal tract or systemically to remove adult helminthes or development froms that invade organs and tissue. They act by either killing (vermin-cides) or by expelling them out[5,8]. 2-Azetidinones are well-known heterocyclic compounds among the organic and medicinal chemists[9-12]. The activity of the famous antibiotics such as penicillins, cephalosporins and carbapenems are attributed to the presence of 2-azetidinone ring in them. Recently, some other types of biological activity besides the antibacterial activity have been reported in compounds containing 2-azetidinone ring[13,14]. Such biological activities include antifungal, antitubercular, antitumor, cholesterol absorption inhibition and enzyme inhibition activity. The β-lactams also serve as synthons for many biologically important classes of organic compounds[15]. Due to this, the investigation of chemistry and biology of these compounds continue to appeal the synthetic and medicinal organic chemists. The most common method for the synthesis of 2-azetidinones is the Staudinger keteneimine cycloaddition, which involves the reaction of imines with acid chloride in the presence of a tertiary base[16].

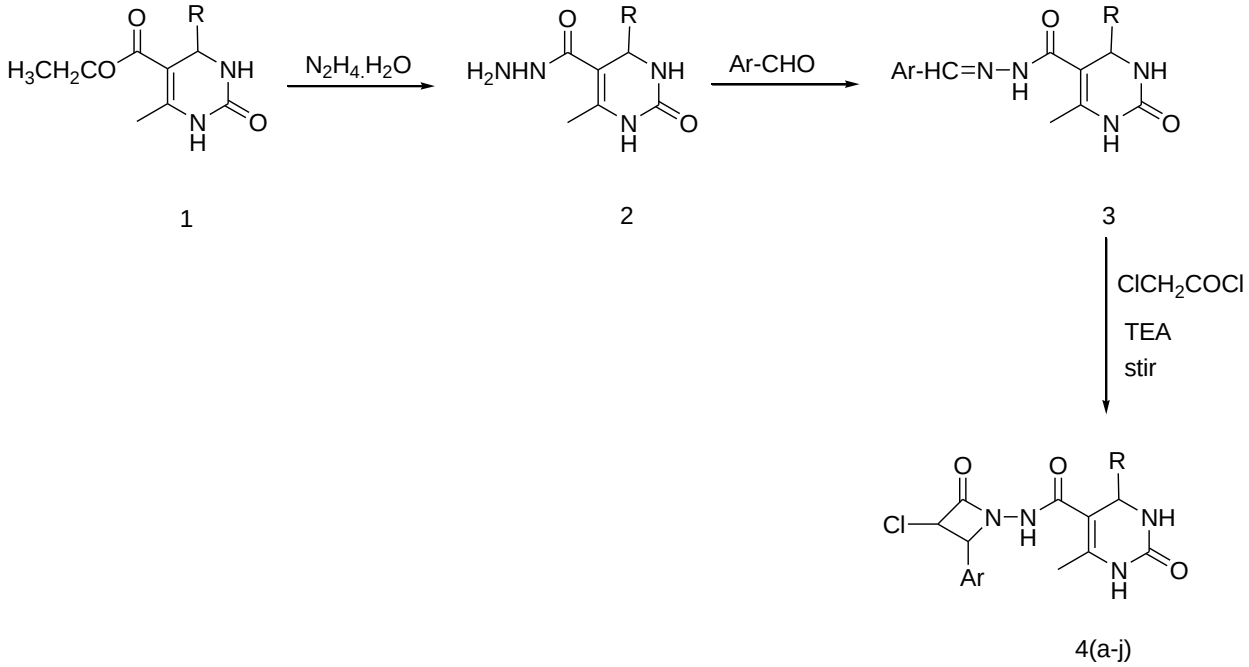
Herein, we have made an effort to synthesize the azetidinone derivatives possessing anthelmintic and anti-bacterial potency.

MATERIALS AND METHODS

All common reagents and solvents were of analytical grade and used directly. Melting points of the synthesized compounds were taken by one end open capillary tube melting point apparatus and are uncorrected. Infra Red (IR) spectra were recorded on Shimadzu FTIR 8400S spectrophotometer (KBr) and ^{1}H NMR spectra were recorded on Bruker-Avance (400 MHz) spectrophotometer with tetramethylsilane (TMS) as an internal standard. *T*hin layer chromatography (TLC) was performed using Silica gel G obtained from Merck and the spots were visualized under iodine vapors.

Method:

Synthesis of ethyl 6-methyl-2-oxo-4-aryl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate *1(a-j)*and corresponding carbohydrazide *2(a-j)* The desired compounds were synthesized as reported in earlier literature[17].



1 2 3

4(a-j)

Scheme 1

where

Compound	R	Ar
a	C_6H_5	3-NO_2- C_6H_4
b	2-OH-C_6H_4	
c	4-OH- C_6H_4	
d	4-OCH_3- C_6H_4	
e	4-N(CH_3)- C_6H_4	
f	C_6H_5	4-Cl- C_6H_4
g	2-OH-C_6H_4	
h	4-OH- C_6H_4	
i	4-OCH_3- C_6H_4	
j	4-N(CH_3)- C_6H_4	

Synthesis of N'-(substituted benzylidene)-1,2,3,4-tetrahydro-6-methyl-2-oxo-4-arylpyrimidine-5-carbohydrazide 3(a-j) 0.01 mol of the carbohydrazide 2(a-j) in absolute ethanol was warmed with 0.01 mol of substituted benzaldehyde with few drops of glacial acetic acid. The reaction completion was monitored by TLC. After completion, the content was poured in ice cold water. The separated product was washed with water, dried and crystallized from ethanol.

Synthesis of N-(3-chloro-2-(substituted phenyl)-4-oxoazetidin-1-yl)-1,2,3,4-tetrahydro-4-aryl-6-methyl-2-oxopyrimidine-5-carboxamide 4(a-j)

0.01M of the above compound mixed with 0.02M triethylamine in absolute ethanol was taken and to it 0.01M chloroacetylcloride was added drop wise at 5-10°C. The reaction mixture was stirred for about 18-20 hr at room temperature. The reaction progress was checked by TLC and after completion; the reaction mixture was poured in ice cold water and further stirred for 1 hr. The product obtained was filtered, dried and recrystallized with ethanol.

Analysis of *N-(3-chloro-2-(3-nitrophenyl)-4-oxoazetidin-1-yl)-1,2,3,4-tetrahydro-6-methyl-2-oxo-4-phenylpyrimidine-5-carboxamide* **4a**

M.pt: 186-189°C. Yield: 68%. IR (cm^{-1}): 2561 (N-N), 1648 (C-C), 3092(Ar C-H), 1576 (C-N), 1711 (C=O), 2944 (C-H). ^{1}H NMR (δ ppm): 1.84 (s, 3H, CH_3), 5.32 (d, 1H, CH), 5.51 (d, 1H, CH), 5.72 (d, 1H, CH), 6.23 (s, 1H, NH), 7.23-8.31 (m, 9H, Ar-H). Anal.Calcd. ($C_{21}H_{18}ClN_5O_5$): C, 55.33; H, 3.98; Cl, 7.78; N, 15.36. Found: C, 55.30; H, 3.94; Cl, 7.73; N, 15.31.

Analysis of N-(3-chloro-2-(3-nitrophenyl)-4-oxoazetidin-1-yl)-1,2,3,4-tetrahydro-4-(2-hydroxyphenyl)-6-methyl-2-oxopyrimidine-5-carboxamide **4b**

M.pt: 182-184°C. Yield: 73%. IR (cm^{-1}): 2582 (N-N), 1678 (C-C), 3091(Ar C-H), 1556 (C-N), 1694 (C=O), 2955 (C-H). ^{1}H NMR (δ ppm): 1.67 (s, 3H, CH_3), 5.21 (d, 1H, CH), 5.69 (d, 1H, CH), 5.81 (d, 1H, CH), 6.80 (s, 1H, NH), 7.01-8.28 (m, 8H, Ar-H). Anal.Calcd. ($C_{21}H_{18}ClN_5O_6$): C, 53.45; H, 3.85; Cl, 7.51; N, 14.84;. Found: C, 53.43; H, 3.79; Cl, 7.47; N, 14.81.

Analysis of N-(3-chloro-2-(3-nitrophenyl)-4-oxoazetidin-1-yl)-1,2,3,4-tetrahydro-4-(4-hydroxyphenyl)-6-methyl-2-oxopyrimidine-5-carboxamide **4c**

M.pt: 177-180°C. Yield: 76%. IR (cm^{-1}): 2572 (N-N), 1685 (C-C), 3067(Ar C-H), 1566 (C-N), 1701 (C=O), 2948 (C-H). ^{1}H NMR (δ ppm): 1.49 (s, 3H, CH_3), 5.26 (d, 1H, CH), 5.58 (d, 1H, CH), 5.74 (d, 1H, CH), 6.37 (s, 1H, NH), 6.54-8.21 (m, 8H, Ar-H). Anal.Calcd. ($C_{21}H_{18}ClN_5O_6$): C, 53.45; H, 3.85; Cl, 7.51; N, 14.84. Found: C, 53.43; H, 3.81; Cl, 7.47; N, 14.78

Analysis of N-(3-chloro-2-(3-nitrophenyl)-4-oxoazetidin-1-yl)-1,2,3,4-tetrahydro-4-(4-methoxyphenyl)-6-methyl-2-oxopyrimidine-5-carboxamide **4d**

M.pt: 181-183°C. Yield: 75%. IR (cm^{-1}): 2568 (N-N), 1655 (C-C), 3070(Ar C-H), 1568 (C-N), 1705 (C=O), 2950 (C-H). ^{1}H NMR (δ ppm): 1.88 (s, 3H, CH_3), 3.65 (s, 3H, O-CH_3), 5.12 (d, 1H, CH), 5.36 (d, 1H, CH), 5.63 (d, 1H, CH), 6.28 (s, 1H, NH), 6.61-8.08 (m, 8H, Ar-H). Anal.Calcd. ($C_{22}H_{20}ClN_5O_6$): C, 54.38; H, 4.15; Cl, 7.30; N, 14.41;. Found: C, 54.32; H, 4.11; Cl, 7.26; N, 14.36.

Analysis of N-(3-chloro-2-(3-nitrophenyl)-4-oxoazetidin-1-yl)-4-(4-(dimethylamino)phenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxamide **4e**

M.pt: 174-177°C. Yield: 66%. IR (cm^{-1}): 2560 (N-N), 1649 (C-C), 3079(Ar C-H), 1561 (C-N), 1710 (C=O), 2948 (C-H). ^{1}H NMR (δ ppm): 1.61 (s, 3H, CH_3), 2.74 (s, 3H, N-CH_3), 5.32 (d, 1H, CH), 5.61 (d, 1H, CH), 5.79 (d, 1H, CH), 6.12 (s, 1H, NH), 6.57-8.14 (m, 8H, Ar-H). Anal.Calcd. ($C_{23}H_{23}ClN_6O_5$): C, 55.37; H, 4.65; Cl, 7.11; N, 16.84. Found: C, 55.33; H, 4.61; Cl, 7.06; N, 16.81.

Analysis of N-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-1,2,3,4-tetrahydro-6-methyl-2-oxo-4-phenylpyrimidine-5-carboxamide **4f**

M.pt: 182-185°C. Yield: 72%. IR (cm^{-1}): 2579 (N-N), 1651 (C-C), 3088(Ar C-H), 1565 (C-N), 1698 (C=O), 2940 (C-H). ^{1}H NMR (δ ppm): 1.58 (s, 3H, CH_3), 4.96 (d, 1H, CH), 5.27 (d, 1H, CH), 5.68 (d, 1H, CH), 6.21 (s, 1H, NH), 6.91-7.82 (m, 9H, Ar-H). Anal.Calcd. ($C_{21}H_{18}Cl_2N_4O_3$): C, 56.64; H, 4.07; Cl, 15.92; N, 12.58. Found: C, 56.56; H, 4.00; Cl, 15.87; N, 12.53.

Analysis of N-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-1,2,3,4-tetrahydro-4-(2-hydroxyphenyl)-6-methyl-2-oxopyrimidine-5-carboxamide **4g**

M.pt:190-191°C. Yield: 77%. IR (cm^{-1}): 2587 (N-N), 1655 (C-C), 3083(Ar C-H), 1560 (C-N), 1709 (C=O), 2949 (C-H). ^{1}H NMR (δ ppm): 1.51 (s, 3H, CH_3), 5.21 (d, 1H, CH), 5.58 (d, 1H, CH), 5.77 (d, 1H, CH), 6.24 (s, 1H, NH), 6.26-7.21 (m, 8H, Ar-H). Anal.Calcd. ($C_{21}H_{18}Cl_2N_4O_4$): C, 54.68; H, 3.93; Cl, 15.37; N, 12.15. Found: C, 54.61; H, 3.90; Cl, 15.33; N, 12.11.

Analysis of N-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-1,2,3,4-tetrahydro-4-(4-hydroxyphenyl)-6-methyl-2-oxopyrimidine-5-carboxamide **4h**

M.pt: 186-189°C. Yield: 69%. IR (cm^{-1}): 2574 (N-N), 1655 (C-C), 3085(Ar C-H), 1576 (C-N), 1713 (C=O), 2956 (C-H). ^{1}H NMR (δ ppm): 1.89 (s, 3H, CH_3), 5.15 (d, 1H, CH), 5.62 (d, 1H, CH), 5.81 (d, 1H, CH), 6.22 (s, 1H, NH), 6.84-7.91 (m, 8H, Ar-H). Anal.Calcd. ($C_{21}H_{18}Cl_2N_4O_4$): C, 54.68; H, 3.93; Cl, 15.37; N, 12.15. Found: C, 54.61; H, 3.90; Cl, 15.33; N, 12.11.

Analysis of N-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-1,2,3,4-tetrahydro-4-(4-methoxyphenyl)-6-methyl-2-oxopyrimidine-5-carboxamide **4i**

M.pt: 188-190°C. Yield: 85%. IR (cm^{-1}): 2580 (N-N), 1643 (C-C), 3076(Ar C-H), 1572 (C-N), 1689 (C=O), 2958 (C-H).^{1}H NMR (δ ppm): 1.82 (s, 3H, CH_3), 3.92 (s, 3H, O-CH_3), 5.20 (d, 1H, CH), 5.60 (d, 1H, CH), 5.84 (d, 1H, CH), 6.17 (s, 1H, NH), 6.51-7.49 (m, 8H, Ar-H). Anal.Calcd. ($C_{22}H_{20}Cl_2N_4O_4$): C, 55.59; H, 4.24; Cl, 14.92; N, 11.79. Found: C, 55.50; H, 4.21; Cl, 14.88; N, 11.74.

Analysis of N-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-4-(4-(dimethylamino)phenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxopyrimidine-5-carboxamide **4j**

M.pt: 192-195°C. Yield: 64%. IR (cm^{-1}): 2581 (N-N), 1652 (C-C), 3081(Ar C-H), 1566 (C-N), 1707 (C=O), 2959 (C-H). ^{1}H NMR (δ ppm): 1.99 (s, 3H, CH_3), 3.01 (s, 3H, N-CH_3), 5.24 (d, 1H, CH), 5.78 (d, 1H, CH), 5.94 (d, 1H, CH), 6.37 (s, 1H, NH), 6.44-7.82 (m, 8H, Ar-H). Anal.Calcd. ($C_{23}H_{23}Cl_2N_5O_3$): C, 56.57; H, 4.75; Cl, 14.52; N, 14.34. Found: C, 56.51; H, 4.69; Cl, 14.47; N, 14.29.

Anthelmintic activity

The Anthelmintic activity was carried out on adult Indian earthworms, *Pheretima postuma*. The worms were collected from local moist place and prior assay; the worms were washed with normal saline so as to remove all fecal matter. They were divided into 12 groups of 6 worms each. Distilled water was used as control while Piperazine citrate (10 mg/ml) was used as reference standard for this study [18-21]. Three different concentrations 5 mg/ml, 10 mg/ml and 15 mg/ml for the compounds and standard drug solution were freshly prepared and poured into Petri dishes. Worms were then introduced into the Petri dishes and observations were made for the time taken for paralysis and death of worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was confirmed when the worms showed zero movement when shaken vigorously or when dipped in warm water (50°C) followed with fading away of their body color. The results obtained are expressed as mean ± S.E.M. (standard error of mean). Statistical differences were carried out using the Analysis of Variance (ANOVA) and was considered significant when $P < 0.05$ Observations obtained are represented in **table 1**.

Table 1 Anthelmintic activity of compounds 4(a-j)

Compounds	5 mg/ml		10 mg/ml		15 mg/ml	
	Time taken for paralysis (min)	Time taken for death (min)	Time taken for paralysis (min)	Time taken for death (min)	Time taken for paralysis (min)	Time taken for death (min)
Control	-	-	-	-	-	-
Piperazine citrate	-	-	21±0.9	59±0.2	-	-
4a	68±0.2	92±0.7	64±0.9	81±0.9	46±1.0	65±0.1
4b	66±0.5	98±0.4*	65±0.7	78±0.3	55±0.6	63±0.5
4c	67±0.4	99±0.7	66±0.5	79±0.6*	51±0.5	62±0.6*
4d	68±0.6	98±0.2	62±0.7	74±0.5	54±0.6	60±0.9
4e	71±0.8*	96±0.5	66±0.1	77±0.2	58±0.9*	59±0.2
4f	70±0.4	81±0.8*	70±1.3	80±1.2	59±0.8	68±0.8
4g	72±0.8	94±0.4	60±0.8	82±0.7	55±0.6	71±0.7
4h	70±0.4	99±0.3	63±0.1*	79±0.9	54±0.3	66±1.2
4i	71±0.5	94±1.2	65±0.2	78±0.8	51±0.5	62±0.4
4j	69±0.7	88±0.5	69±0.6	73±1.3	52±0.7	70±0.7

Results were given in mean ± SEM and analyzed by ANOVA

∗ P < 0.05 compared to standard drug.

Anti-bacterial activity

The in-vitro anti-bacterial activity of the newly synthesized compounds **4(a-h)** was estimated by the well diffusion method using Hi-Media agar medium against *S. aureus, B. subtilis, E. coli* and *K. pneumoniae* strains of bacteria. For the analysis, Petri plates of agar medium were prepared by pouring melted agar inoculated with above mentioned strains of bacteria. Wells were scooped out of the agar medium contained in these Petri plates. Sample size for all the compounds was fixed at 0.1 ml. The test compound solution (0.1 ml) was added in the wells and the Petri plates were subsequently incubated at 37°C for 24 hr. Ampicillin and Streptomycin were used as reference drugs and ethanol as the negative control. The zones of inhibition thus produced by each compound were measured and compared with the control and the consequent results are depicted in **table 2.**

Table 2: Antibacterial Activity of Compounds 4(a-j)

Compounds	**Gram-positive bacteria**		**Gram-negative bacteria**	
	S. aureus	*B. subtilis*	*E. coli*	*K. pneumoniae*
4a	++	+	++	++
4b	++	+++	++	+
4c	+	++	-	++
4d	++	+++	++	+++
4e	+++	+	++	+++
4f	+	++	+	++
4g	++	+++	+++	++
4h	+	-	-	+
4i	++	+++	+++	++
4j	+++	+	++	-
Ampicillin	+++	++	+++	++
Streptomycin	+++	+++	+++	+++

Key to symbols: inactive = - (inhibition zone < 5 mm); slightly active = + (inhibition zone 5-10 mm); moderately active = + + (inhibition zone 10-15 mm); highly active = + + + (inhibition zone > 15 mm).

RESULTS AND DISCUSSION

Various azetidinone derivatives using different aldehydes have been synthesized herein as per the synthetic protocol and reaction sequence sketched out in Scheme 1. The products were obtained in 64-85% yield. The identity of the products obtained was confirmed on the basis of their elemental analysis and spectral data. The IR spectra of these compound gave prominent peaks at 1713-1689 cm^{-1} (C=O), 2587-2560 cm^{-1} (N-N) stretching. ^{1}H NMR spectra of these derivatives gave multiplet

for aromatic protons between 6.26- 8.31ppm. The other signals and peaks of IR ^{1}H and NMR are comparitively specific with the assigned structures. The compounds gave acceptable results for their elemental analysis. The synthesized compounds were also tested for their in vitro anthelmintic at three different concentrations 5 mg/ml, 10 mg/ml and 15 mg/ml for which acceptable activity was obtained. Moreover, in vitro anti-bacterial activity for the synthesized compounds was also evaluated against some gram positive and gram negative strains of bacteria using the well diffusion method. Amongst the synthesized compounds, compounds **4d, 4g** and **4i** showed broad spectrum of antibacterial activity with acceptable zone of inhibition for all the tested strains of bacteria while **4c** and **4h** showed less activity against the selected pathogens.

CONCLUSION

A series of pyrazolone derivatives were synthesized in appreciable yield. The compounds showed moderate to good results for the anthelmintic activity analysis at concentrations of 5 mg/ml, 10 mg/ml and 15 mg/ml when compared to the standard reference drug Piperazine citrate. The compounds were also found to be the active antibacterial agents with reference to standard drug Ampicillin and Streptomycin at a dose of 1000 µg/ml and thus these synthesized compounds can be used as anti-inflammatory and anti-bacterial agents.

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