Synthesis and Medicinal Evaluation of Mannich Bases Carrying Azetidinone Moiety

Azetidinon Payı Taşıyan Mannik Bazların Sentezi ve Tıbbi Değerlendirmesi

Mannik Bazların Sentezi ve Tıbbi Değerlendirmesi / Synthesis and Medicinal Evaluation of Mannich Bases

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Özet

Amaç: Azatidinon payı taşıyan Mannik bazlar serisini sentezlemek ve antibakteriyel, antifungal, antihelmintik activitilerini in vitro olarak taramak ve karakterize etmek. Gereç ve Yöntem: Paylar IR, ^{1}H NMR ve kitle spektra ile ve elemental analiz ile karakterize edildiler. Seçilen ajana yönelik antimikrobiyal aktiviteleri disk difüzyon yöntemi ile tespit edildi. Antihelmintik aktivite çalışmaları Pheretima posthuma ile gerçekleştirildi. Bulgular: Maddelerin antimikrobiyal aktiviteleri siprofloksasin aktivitesi ile antifungal aktiviteleri klotrimazol aktivitesi ile ve antihelmintik aktiviteleri piperazin sitrat ile karşılıştırıldı. Tartışma: Tüm maddelerin antimikrobiyal aktivite gösterdiği izlenirken özellikle 7c, 7e, 7f ve 7h ürünlerinin aktiviteleri çalışmada uygulanan standart antibiyotiklerle karşılaştırılabilir idi. Ayrıca, N-metilpiperazin parçası içeren madde 7h standart ilaç ile karşılaştırılabilir antihelmintik aktivite göstermiştir.

Anahtar Kelimeler

Mannik Bazlar; Azetidinon Payı; Antimikrobiyal Aktivite; Antihelmintik Aktivite

Abstract

Aim: To synthesize, characterize and in vitro screen for antibacterial, antifungal and anthelmintic activity of series of Mannich bases carrying azetidinone moiety. Material and Method: The compounds were characterized by IR, ^{1}H NMR and mass spectra and by elemental analysis. Their antimicrobial activity against the selected microorganism was found out by disk diffusion method. Anthelmintic activity studies were performed on Pheretima posthuma. Results: The antibacterial activity of the title compounds was compared with that of ciprofloxacin, antifungal activity with clotrimazole and anthelmintic activity was compared with that of piperazine citrate. Discussion: All the compounds showed significant antimicrobial activity in particular, the activity of compounds 7c, 7e, 7f and 7h was comparable to that of standard antibiotics employed in the studies. Further, the compound 7h with N-methylpiperazine substituent showed anthelmintic activity comparable to that of the standard drug.

Keywords

Mannich Bases; Azetidinone Moiety; Antimicrobial Activity; Anthelmintic Activity

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Introduction

The simplest β-lactam possible is 2-azetidinone in which the nitrogen atom is attached to the β-carbon relative to the carbonyl group and is a part of the several β-lactam antibiotics namely penicillins, cephalosporins, carbapenems and monobactams. The 2-azetidinone template has been widely described as a lead structure for a wide spectrum of pharmacological activities. Hence the development of new synthetic methodology incorporating the β-lactam nucleus referred as β-lactam synthon method is a vigorous research approach in the current scenario. The activity of β-lactam antibiotics depends on the irreversible inhibition of bacterial cell wall synthesis. In particular, the D-alanyl-transpeptidase necessary for the murein synthesis of bacterial cell wall is inhibited [1]. Particularly a large number of 3-chloro monocyclic ß-lactams exhibit significant antimicrobial, anti-inflammatory, anticonvulsant, antitubercular etc activities [2-7]. They also function as enzyme inhibitors and are effective on the central nervous system [8, 9]. Due to the diverse nature of this scaffold, namely 2-azetidinones and in continuation of our search for novel pharmacologically active derivates [10-13], we herein report the synthesis and pharmacological evaluation of title compounds.

Material and Method

All chemicals and regents were procured from Merck India Limited. Nutrient broth, nutrient agar and 5 mm diameter antibiotic assay discs were obtained from Hi-Media Laboratories Limited, India. The standard bacterial and fungal strains were procured from National Centre for Cell Sciences, Pune, India. Synthesized compounds were recrystallized using suitable solvent. Melting points were determined by Scientific melting point apparatus, India and were uncorrected. Digital electronics balance (Shankar Scientific supplies, India), horizontal laminar air flow bench (Yorco sales Pvt. Ltd, New Delhi, India), incubator (Yorco sales Pvt. Ltd, New Delhi, India), zone reader (Cintex Industrial Corporation, India) and hot air oven were used for relevant investigations. Infrared spectra of the compounds were recorded in KBr discs on Perkin-Elmer FT IR spectrometer (υ_{max} in cm^{-1}). ^1H NMR spectra were recorded on a JOEL (300 MHz) spectrometer using TMS as an internal standard (chemical shifts in δ).

Estimation of antimicrobial activity by Disc Diffusion Method

A suspension of Staphylococcus aureus was added to sterile nutrient agar at 45° C. The mixture was transferred to sterile petridishes to give a depth of 3 to 4 mm and allowed to solidify. Sterile discs of 5 mm diameter (Whatmann Filter paper) were immersed in solutions of synthesized compounds (20 µg/mL) and untreated control sample was also prepared for comparison.

A period of pre incubation diffusion (1 hour at room temperature) was ensured to minimize the effects of variations in time. The plates were incubated at 37° C for 24 hours and observed for antibacterial activity. The diameter of the zone of inhibition was measured for each plate in which the zone of inhibition was observed. The average zone of inhibition was calculated and compared with that of the standard. Similar procedure was adopted for studying the antimicrobial activity against the other organisms.

Procedure for anthelmintic studies

Adult earthworm, Pheretima posthuma, was collected from moist soil and washed with double distilled water. The earthworms of 5-6 cm in length were used in present investigations. Test samples were prepared at concentrations of 20 mg/mL in dimethylformamide. Six worms i.e. Pheretima posthuma, were placed in petri dish containing 50 mL of test solution. Piperazine citrate (10 mg/mL) was used as reference standard. Time corresponding to paralysis and death of the worm were determined.

Results and Discussion

Synthesis of ethyl 4,4,4-trichloro-3-oxo-2-(2-(4-substituted) phenyl hydrazono) butanoate (1)

Ethyl-4,4,4-trichloro-3-oxo-2-(2-phenyl hydrazono)-butanoate (1) was prepared by the procedure described by H.M.W.Alborsky and M.E.Baum [14].

Synthesis of 4-(2-(4-substituted)phenyl hydrazono)-3-(trichloro methyl)-1H-pyrazol-5(4H)-one (2)

A mixture of (1, 0.02 M), hydrazine hydrate (10 mL) and dimethyl formamide (10 drops) was subjected to microwave irradiation at 150 W intermittently at 30 seconds intervals for 2 minutes. After complete conversion as indicated by TLC, the reaction mixture was cooled and treated with cold water. The precipitated 3-methyl 4-(phenylhydrazono) pyrazoline-5-one (2a) was filtered and recrystallized from ethanol. Similar procedure was adopted for the synthesis of other compounds of the series 2b-f.

Synthesis of [5-Oxo-4-(4-substituted aryl hydrazono)-3-trichloro methyl-4,5-dihydro- pyrazol-1- yl]-acetic acid ethyl ester (3)

A mixture of (2) (0.02M), anhydrous K_2CO_3 (0.03M), chloro ethyl acetate (0.02M) and DMF was stirred at room temperature for 8 hours. The progress of the reaction was monitored by TLC using cyclohexane:ethylacetate (9:1) as elutent. After completion of the reaction, the reaction mixture was diluted with ice cold water. The solid separated was collected by filtration, identified as [5-Oxo-4-(phenyl hydrazono)-3-trichloromethyl-4, 5-dihydro-pyrazol-1- yl]-acetic acid ethyl ester (3a) and recrystallized from ethanol. Similar procedure was followed for the synthesis of other compounds 3b-f.

Synthesis of Ethyl 2-(3-chloro-2,5-dioxo-1-(phenylamino)-8-(trichloromethyl)-1,6,7-triazaspiro[3.4]oct-7-en-6-yl)acetate (4a)

A solution of monochloroacetyl chloride (0.01 M, 20 mL) was added drop wise to a mixture of schiff's base (3) (0.01 M), triethyl amine (0.02 M) in dioxane (25 mL) at room temperature. The progress of the reaction was monitored by TLC using cyclohexane:ethylacetate (9:1) as elutent. After completion of the reaction, the mixture was stirred for 8 hours and left undisturbed at room temperature for 3 days. The contents were poured on crushed ice, filtered and washed with sodium bicarbonate solution. The dried product was recrystallized from absolute alcohol. Similar procedure was adopted for the synthesis of other compounds 4b-f. The structures of the newly synthesized compounds were established by elemental, IR and ^1H NMR spectral analysis.

IR(KBr) spectrum of ethyl 2-(3-chloro-2,5-dioxo-1-(phenylamino)-8-(trichloromethyl)-1,6,7-triazaspiro[3.4]oct-7-en-6-yl)acetate (4a) shows signals at around 3340 cm^{-1}, 1690 cm^{-1} and 677 cm^{-1} due to stretching vibrations of –OH, >C=O, C-Cl respectively.

^{1}H NMR Spectrum of 4a shows signals at 4.6 (s, 1H, -OH), 5.16 (d, 1H, -CH of azetidin attached to phenyl ring), 5.44 (d, 1H, -CH of azetidin attached to –Cl), 6.57 (d, 1H, -CH), 6.76 (d, 1H, -CH), 7.12-7.21 (m, 5H of C_6H_5), 7.9-8.8 (m, 3H of quinoline ring).

Synthesis of 2-(3-chloro-2,5-dioxo-1-(4-substituted phenylamino)-8-(trichloromethyl)-1,6,7-triazaspiro[3.4]oct-7-en-6-yl)acetohydrazide (5a-f)

A mixture of ethyl 2-(3-chloro-2,5-dioxo-1-(phenylamino)-8-(trichloromethyl)-1,6,7-triazaspiro[3.4]oct-7-en-6-yl)acetate (4a) (0.01 M) and hydrazine hydrate (0.015 M) in ethanol (20 mL) was refluxed for 5 hours. The reaction mixture was cooled and poured on to ice cold water with stirring. The progress of the reaction was monitored by TLC with acetone:ethyl acetate (7:3) as eluent. The solid separated was filtered, washed with water and recrystallized from ethanol to give 2-(3-chloro-2,5-dioxo-1-(4-substituted phenylamino)-8-(trichloromethyl)-1,6,7-triazaspiro[3.4]oct-7-en-6-yl)acetohydrazide.

IR(KBr) spectrum of 2-(3-chloro-2,5-dioxo-1-(phenylamino)-8-(trichloromethyl)-1,6,7-triazaspiro[3.4]oct-7-en-6-yl)acetohydrazide (5a) shows signals at 3320 cm^{-1}, 1660 cm^{-1} and 687 cm^{-1} due to stretching vibrations of –OH, >C=O, C-Cl respectively.

^{1}H NMR spectrum of 5a shows signals at 4.5 (s, 1H, -OH), 5.19 (d, 1H, -CH of azetidin attached to phenyl ring), 5.39 (d, 1H, -CH of azetidin attached to –Cl), 6.67 (d,1H,-CH), 6.52 (d, 1H, -CH), 7.01-7.17 (m, 5H of C_6H_5), 7.74-8.66 (m, 3H of quinoline ring).

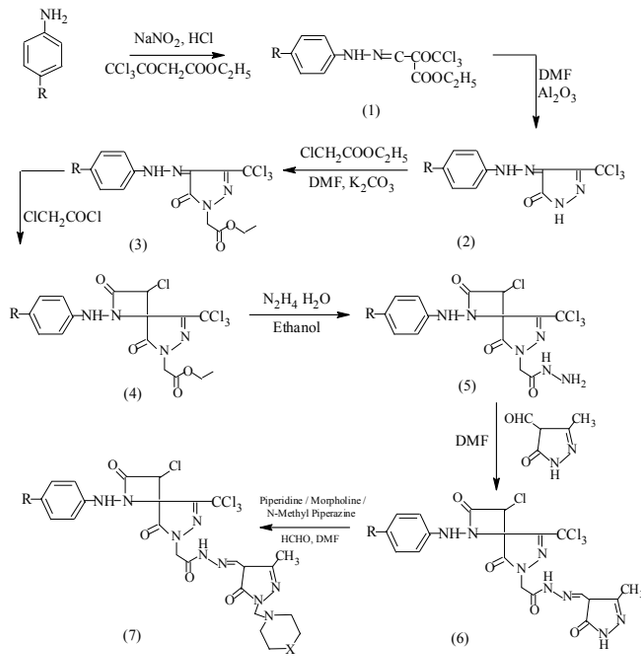
Synthesis of 2-(3-chloro-2,5-dioxo-1-(4-substituted phenylamino)-8-(trichloromethyl)-1,6,7-triazaspiro[3.4]oct-7-en-6-yl)-N'-((3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl) methylene)acetohydrazide (6a-f)

Equimolar quantities (0.01 M) of 3-methyl-5-oxo-4,5-dihydro-1H-pyrazole-4-carbaldehyde and the corresponding amino compound (5a-f) were dissolved in warm ethanol (40 mL) containing DMF (0.5 mL). The reaction mixture was refluxed for 1-4 hours and then kept overnight at room temperature. The progress of the reaction was monitored by TLC using cyclohexane:ethylacetate (9:1) as an eluent. After completion of the reaction, the resulting solid was filtered, washed with ethanol, dried and recrystallized from ethanol to afford compounds 6a-f.

Synthesis of 2-(3-chloro-2,5-dioxo-1-(4-substituted phenylamino)-8-(trichloromethyl)-1,6,7-triazaspiro[3.4]oct-7-en-6-yl)-N'-((3-methyl-5-oxo-1-(morpholin/piperidin/N-methylpiperazine-1-ylmethyl)-4,5-dihydro-1H-pyrazol-4-yl)methylene)acetohydrazide (7a-h)

A mixture of (6a) (0.1 M), morpholine (0.15 M) and water (20 mL) was thoroughly stirred to obtain a clear solution. HCHO and DMF (0.05 M each, 3 mL) in ethanol were added to the above mixture at 0°C, stirred for 2 hours in an ice bath and left overnight at room temperature. The white solid obtained was isolated and recrystallized from ethanol to give Compound (7a).

The reaction procedure leading to 7a was extended for the syntheses of (7b-h).



Scheme-VII

Scheme 1. Synthesis of 2-(3-chloro-2,5-dioxo-1-(4-substituted phenylamino)-8-(trichloromethyl)-1,6,7-triazaspiro[3.4]oct-7-en-6-yl)-N'-((3-methyl-5-oxo-1-(morpholin/piperidin/N-methylpiperazine-1-ylmethyl)-4,5-dihydro-1H-pyrazol-4-yl)methylene)acetohydrazide (7a-h)

Comp	7a	7b	7c	7d	7e	7f	7g	7h
R	H	4-CH_3	4-OCH_3	4-OC_2H_5	4-Cl	4-Br	H	H
X	O	O	O	O	O	O	CH_2	N-CH_3

Characterisation of 2-(3-chloro-2,5-dioxo-1-(4-substituted phenylamino)-8-(trichloromethyl)-1,6,7-triazaspiro[3.4]oct-7-en-6-yl)-N'-((3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl) methylene)acetohydrazide (6a-f)

Compound: m.p. °C; Yield (%); Molecular formula; Elemental Analysis: Element Found% (Calculated%); IR spectral data: IR vmax in cm^{-1}(Group); ^{1}H NMR spectral data: (300 MHz, DMSO-d_6) δ ppm.

6a: 198-9; 69; $C_{19}H_{16}Cl_4N_8O_4$; C 40.51 (40.59), H 2.95 (2.87), N 19.88 (19.93); 3222 (NH of pyrazoline), 1690 (C=O of azetidine), 1592 (C=N), 687 (C-Cl of azitidine); 1.98(s, 3H, -CH_3 attached to pyrazolone), 4.05 (s, 2H, -$COCH_2$), 5.44(d, 1H, -CH of azetidine attached to Cl), 6.92-7.39(m, 5H, C_6H_5), 7.05(s, 1H, pyrazolone NH), 7.52(d, 1H, =CH-), 8.09(s, 1H, hydrazide NH), 9.04(s, 1H, Ar-NH).

6b: 206-7; 61; $C_{20}H_{18}Cl_4N_8O_4$; C 41.65 (41.69), H 3.18 (3.15), N 20.41 (19.45); 3225 (NH of pyrazoline), 1660 (C=O of azetidine), 1684 (C=N), 690 (C-Cl of azitidine); 1.81(s, 3H, -CH_3 attached to pyrazolone), 2.34 (s, 3H, -CH_3), 4.12 (s, 2H, -$COCH_2$), 5.34(d, 1H, -CH of azetidine attached to Cl), 6.92-7.39(m, 4H, C_6H_4), 7.05(s, 1H, pyrazolone NH), 7.50(d, 1H, =CH-), 7.99(s, 1H, hydrazide NH), 8.57(s, 1H, Ar-NH).

6c: 212-3; 60; $C_{20}H_{18}Cl_4N_8O_5$; C 40.02 (40.56), H 3.01 (3.06), N 20.92 (18.95); 3223 (NH of pyrazoline), 1656 (C=O of azetidine), 1688 (C=N), 687 (C-Cl of azitidine); 1.88(s, 3H,-CH_3 attached to pyrazolone), 3.83 (s, 3H, -OCH_3), 4.09 (s, 2H, -$COCH_2$), 5.61(d, 1H,

CH of azetidine attached to Cl), 6.72-6.91(m, 4H, C_6H_4), 7.07(s, 1H, pyrazolone NH), 7.55(d, 1H, =CH-), 8.01(s, 1H, hydrazide NH), 8.81(s, 1H, Ar-NH).

6d: 217-8; 59; $C_{21}H_{20}Cl_4N_8O_5$; C 41.29 (41.60), H 3.36 (3.33), N 18.4 4 (18.48); 3218 (NH of pyrazoline), 1648 (C=O of azetidine), 1689 (C=N), 685 (C-Cl of azitidine); 1.32 (q, 3H, -CH_3 of ethoxy), 1.77(s, 3H, -CH_3 attached to pyrazolone), 4.07 (t, 2H, -OCH_2 of ethoxy), 4.01(s, 2H, -$COCH_2$), 5.53(d, 1H, CH of azetidine attached to Cl), 6.69-6.89(m, 4H, C_6H_4), 7.08(s, 1H, pyrazolone NH), 7.56(d, 1H, =CH-), 8.02(s, 1H, hydrazide NH), 8.73(s, 1H, Ar-NH).

6e: 197-8; 65; $C_{19}H_{15}Cl_5N_8O_4$; C 38.52 (38.25), H 2.55 (2.53), N 18.89 (18.78); 3228 (NH of pyrazoline), 1645 (C=O of azetidine), 1679 (C=N), 692 (C-Cl of azitidine); 2.01(s, 3H,-CH_3 attached to pyrazolone), 4.08 (s, 2H, -$COCH_2$), 5.50(d, 1H, CH of azetidine attached to Cl), 6.76-7.39(m, 4H, C_6H_4), 7.12(s, 1H, pyrazolone NH), 7.62(d, 1H, =CH-), 8.00(s, 1H, hydrazide NH), 9.05(s, 1H, Ar-NH).

6f: 202-3; 58; $C_{19}H_{15}Br\ Cl_4N_8O_4$; C 35.59 (35.60), H 2.31 (2.36), N 17.41 (17.48); 3220 (NH of pyrazoline), 684 (C=O of azetidine), 1647 (C=N), 683 (C-Cl of azitidine); 2.09(s, 3H,-CH_3 attached to pyrazolone), 4.08 (s, 2H, -$COCH_2$), 5.51(d, 1H, CH of azetidine attached to Cl), 6.73-7.49(m, 4H, C_6H_4), 7.11(s, 1H, pyrazolone NH), 7.61(d, 1H, =CH-), 8.01(s, 1H, hydrazide NH), 9.05(s, 1H, Ar-NH).

Characterisation of 2-(3-chloro-2,5-dioxo-1-(4-substituted phenylamino)-8-(trichloromethyl)-1,6,7-triazaspiro[3.4]oct-7-en-6-yl)-N'-((3-methyl-5-oxo-1-(morpholin/piperidin/N-methylpiperazine-1-ylmethyl)-4,5-dihydro-1H-pyrazol-4-yl)methylene)acetohydrazide (7a-h)

Compound: m.p. °C; Yield (%); Molecular formula; Elemental Analysis: Element Found% (Calculated%); IR spectral data: IR ν_{max} in cm^{-1}(Group); ^1H NMR spectral data: (300 MHz, DMSO-d_6) δ ppm.

7a: 195-6; 61; $C_{24}H_{25}Cl_4N_9O_5$; C 43.53 (43.59), H 3.83 (3.81), N 19.11 (19.06); 3205 (Ar-NH), 1690 (C=O of azitidinone), 1617 (C=O of pyrazolone), 1592 (C=N), 687 (C-Cl of azitidine); 1.89(s, 3H, -CH_3 attached to pyrazolone), 3.91 (s, 2H, -$COCH_2$), 5.64(d, 1H,- CH of azetidine attached to –Cl), 6.92-7.39(m, 5H, C_6H_5), 7.59(d, 1H, =CH), 7.97(s, 1H, hydrazide NH), 8.89(s, 1H, Ar-NH), 3.52 (t, 4H, -CH_2-O-CH_2 of morpholine ring), 2.45 (t, 4H, -CH_2-N-CH_2 of morpholine ring), 4.85 (s, 2H, -N-CH_2-N-).

7b: 214-5; 58; $C_{25}H_{27}Cl_4N_9O_5$; C 44.41 (44.46), H 4.06 (4.03), N 18.61 (18.67); 3206 (Ar-NH), 1660 (C=O of azitidinone), 1616 (C=O of pyrazolone), 1684 (C=N), 690 (C-Cl of azitidine); 1.79(s, 3H, -CH_3 attached to pyrazolone), 2.34 (s, 3H, -CH_3), 4.03 (s, 2H, -$COCH_2$), 5.49(d, 1H, -CH of azetidine attached to Cl), 6.75-7.12(m, 4H, C_6H_4), 6.92-7.39(m, 5H, C_6H_5), 7.53(d, 1H, =CH), 7.91(s, 1H, hydrazide NH), 8.61(s, 1H, Ar-NH), 3.51 (t, 4H, -CH_2-O-CH_2 of morpholine ring), 2.46 (t, 4H, -CH_2–N–CH_2 of morpholine ring), 4.86 (s, 2H, -N-CH_2-N-).

7c: 219-0; 54; $C_{25}H_{27}Cl_4N_9O_6$; C 43.39 (43.43), H 3.91 (3.94), N 18.19 (18.23); 3208 (Ar-NH), 1656 (C=O of azitidinone), 1621 (C=O of pyrazolone), 1688 (C=N), 687 (C-Cl of azitidine); 1.86(s, 3H, -CH_3 attached to pyrazolone), 3.83 (s, 3H, -OCH_3), 4.01 (s, 2H, -$COCH_2$), 5.68(d, 1H, -CH of azetidine attached to Cl), 6.72-6.91(m, 4H, C_6H_4), 7.60(d, 1H, =CH), 7.95(s, 1H, hydrazide NH), 8.51(s, 1H, Ar-NH), 3.50 (t, 4H, -CH_2-O-CH_2 of morpholine ring), 2.42 (t, 4H, -CH_2-N-CH_2 of morpholine ring), 4.83 (s, 2H, –N–CH_2–N–).

7d: 222-3; 45; $C_{26}H_{29}Cl_4N_9O_6$; C 44.22 (44.27), H 4.17 (4.14), N 17.81 (17.87); 3210 (Ar-NH), 1648 (C=O of azitidinone), 1618 (C=O of pyrazolone), 1689 (C=N), 685 (C-Cl of azitidine); 1.32 (q, 3H, -CH_3 of ethoxy), 1.71(s, 3H,-CH_3 attached to pyrazolone), 4.06 (s, 2H, -$COCH_2$), 4.07 (t, 2H, -OCH_2 of ethoxy), 5.69(d, 1H, -CH of azetidine attached to Cl), 6.69-6.89(m, 4H, C_6H_4), 7.62(d, 1H, =CH), 7.94(s, 1H, hydrazide NH), 8.49(s, 1H, Ar-NH), 3.51 (t, 4H, -CH_2-O-CH_2 of morpholine ring), 2.42(t, 4H, -CH_2-N-CH_2 of morpholine ring), 4.84 (s, 2H, -N-CH_2-N-).

7e: 234-5; 50; $C_{24}H_{24}Cl_5N_9O_5$; C 41.39 (41.43), H 3.54 (3.48), N 18.15 (18.12); 3209 (Ar-NH), 1645 (C=O of azitidinone), 1620 (C=O of pyrazolone), 1679 (C=N), 692 (C-Cl ofazitidine); 1.93(s, 3H, -CH_3 attached to pyrazolone), 4.10 (s, 2H, -$COCH_2$), 5.59(d, 1H, -CH of azetidine attached to Cl), 6.76-7.39(m, 4H, C_6H_4), 7.69(d, 1H, =CH), 7.90(s, 1H, hydrazide NH), 8.93(s, 1H, Ar-NH), 3.49 (t, 4H, -CH_2-O-CH_2 of morpholine ring), 2.43 (t, 4H, -CH_2-N-CH_2 of morpholine ring), 4.81 (s, 2H, -N-CH_2-N-).

7f: 238-9; 51; $C_{24}H_{24}BrCl_4N_9O_5$; C 38.91 (38.94), H 3.29 (3.27), N 17.06 (17.03); 3210 (Ar-NH), 1684 (C=O of azitidinone), 1615 (C=O of pyrazolone), 1647 (C=N), 684 (C-Cl of azitidine); 2.01(s, 3H, -CH_3 attached to pyrazolone), 4.09 (s, 2H, -$COCH_2$), 5.61(d, 1H, -CH of azetidine attached to Cl), 6.73-7.49(m, 4H of C_6H_4), 7.65(d, 1H, =CH), 7.89(s, 1H, hydrazide NH), 8.92(s, 1H, Ar-NH), 3.50 (t, 4H, -CH_2-O-CH_2 of morpholine ring), 2.43 (t, 4H, -CH_2-N-CH_2 of morpholine ring), 4.81 (s, 2H, -N-CH_2-N-).

7g: 201-2; 50; $C_{25}H_{27}Cl_4N_9O_4$; C 45.59 (45.54), H 4.20 (4.13), N 19.17 (19.12); 3205 (Ar-NH), 1690 (C=O of azitidinone), 1617 (C=O of pyrazolone), 1592 (C=N), 687 (C-Cl of azitidine); 1.30–1.51 (m, 6H $(CH_2)_3$ of piperidine ring), 2. 10 (t, 4H, -CH_2-N-CH_2 of piperidine ring), 4.85 (s, 2H, -N-CH_2-N-), 1.98(s, 3H,-CH_3 attached to pyrazolone), 4.05 (s, 2H, -$COCH_2$), 5.44 (d, 1H, -CH of azetidine attached to Cl), 6.92-7.39(m, 5H of C_6H_5), 7.52(d, 1H, =CH), 8.09 (s, 1H, hydrazide NH), 9.04(s, 1H, Ar-NH), 3.52 (t, 4H, –CH_2–O–CH_2 of morpholine ring), 2.45 (t, 4H, -CH_2-N-CH_2 of morpholine ring), 4.85 (s, 2H, -N-CH_2-N-).

7h: 219-0; 55; $C_{25}H_{28}Cl_4N_{10}O_4$; C 44.49 (44.53), H 4.22 (4.19), N 20.81 (20.77); 3205 (Ar-NH), 1690 (C=O of azitidinone), 1617 (C=O of pyrazolone), 1592 (C=N), 687 (C-Cl of azitidine); 2.26 (s, 3H, N-CH_3), 2.45 (t, 4H, -CH_2-N-CH_2 of piperazine ring), 4.75 (s, 2H, -N-CH_2–N-), 2.32 (t, 4H, -CH_2-N(CH_3)-CH_2, 1.98(s, 3H, -CH_3 attached to pyrazolone), 4.00 (s, 2H, -$COCH_2$), 5.41(d, 1H, -CH of azetidine attached to Cl), 6.72-7.15(m, 5H of C_6H_5), 7.52(d, 1H, =CH), 8.09(s, 1H, hydrazide NH), 9.01(s, 1H, Ar-NH).

Antimicrobial activity

The antimicrobial activity of compounds was explored by the disc diffusion method [15] against selected microorganisms. The gram positive bacteria screened were Staphylococcus aureus NCCS 2079 and Bacillus cereus NCCS 2106. The gram negative bacterial screened were Escherichia coli NCCS 265 and Pseudomonas aeruginosa NCCS2200. The fungi screened were Aspergillus niger nccs 1196 and Candida albicans NCCS 3471.

The results shown in Figure 1 revealed that all compounds were active against the tested microbes and demonstrated high to moderate activity as compared to standards. It was interest-

ing to note that among the compounds synthesized, compounds 7c, 7e, 7h and 7f were more sensitive to the tested organisms. Hence it can be inferred that the presence of halogen group and methoxy group at 4 position i.e. compounds 7c, 7e and 7h exhibited potent antimicrobial activity. Also presence of N-CH_3 group in piperazine ring i.e compound 7f has exhibited remarkable antimicrobial and anthelmintic activity. The result pertaining to anthelmintic activity has once again witnessed the anthelmintic action of entire class of piperazine-containing compounds.

Anthelmintic activity

Anthelmintic activity studies were performed on Pheretima posthuma. The results are given in their Table 1.The selection of Pheretima posthuma for anthelmintic studies is owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [16-18]. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded after ascertaining that worms did not move when shaken vigorously or even when dipped in warm water (at 50 °C) followed with fading away of their body colors.

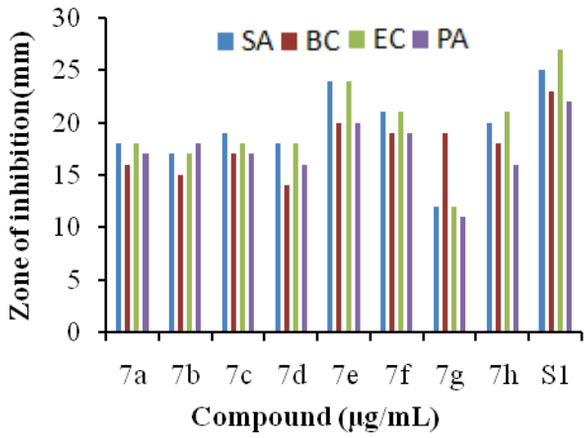


Figure 1. Antibacterial activity of Mannich bases

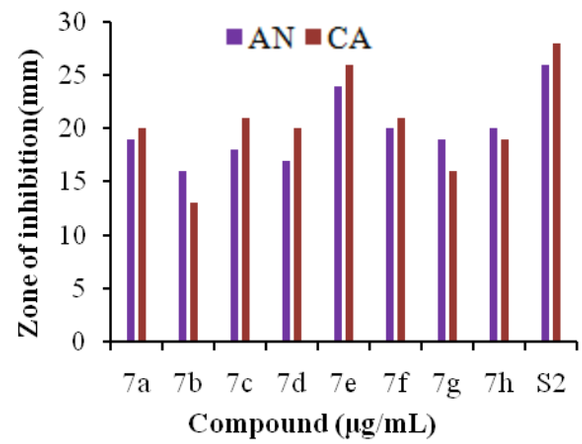


Figure 2. Antifungal activity of Mannich bases

SA: Staphylococus aureus NCCS 2079; BC: Bacillus Cereus NCCS 2106; EC: Escherichia coli NCCS 2065; PA: Pseudomanas aeruginos NCCS 2200; AN: Aspergillus niger NCCS 1196; CA: Candida albicans NCCS 2106, S1: Ciprofloxacin; S2: Clotrimazole.

Table 1. Anthelmintic activity studies

Anthelmintic activity (minute)	Piperazine citrate (10 mg/mL)	7a	7b	7c	7d	7e	7f	7g	7h
		20 mg/mL							
Paralysis	28	--	--	--	--	--	--	--	29
Death	63	--	--	--	--	--	--	--	65

Conclusion

A series of eight novel 2-(3-chloro-2,5-dioxo-1-(4-substituted phenylamino)-8-(trichloromethyl)-1,6,7-triazaspiro[3.4]oct-7-en-6-yl)-N'-((3-methyl-5-oxo-1-(morpholin/piperidin/N-methylpiperazine-1-ylmethyl)-4,5-dihydro-1H-pyrazol-4-yl)methylene) acetohydrazides has been synthesized and characterized. While all the novel compounds were active against certain bacteria and fungi, the compounds 7c, 7e, 7f and 7h were found to exhibit significant antimicrobial activity . It was also found that the compound 7h containing N-methylpiperazine moiety showed remarkable anthelmintic activity.

Competing interests

Authors declare that there are no competing interests concerning this article.

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