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Synthesis, Analgesic and Anti-inflammatory Evaluation of some Novel Azetidinones

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Abstract

A series of nine novel azetedinones **2a-i** have been synthesized by condensation of 2, 4-dinitro phenylhydrazine with various substituted aromatic aldehydes in the presence of zinc chloride and methanol followed by the reaction with chloroacetyl chloride and triethylamine. The synthesized compounds were characterized by IR, ^{1}H-NMR and elemental analysis. The titled compounds were evaluated for analgesic and anti-inflammatory activity by Eddy's hot plate method and carrageenan induced paw edema method respectively. Compounds 3-chloro-1-(2,4-dinitrophenylamino)-4-(3-hydroxy-4-methoxyphenyl)azetidin-2-one, 3-chloro-1-(2,4-dinitrophenylamino)-4-(4-hydroxyphenyl) azetidin-2-one and 3-chloro-4-(2,4-dichlorophenyl)-1-(2,4-dinitrophenylamino) azetidin-2-one showed significant analgesic activity by increasing the reaction time. Compounds 3-chloro-1-(2,4-dinitrophenylamino)-4-(3-hydroxy-4-methoxyphenyl)azetidin-2-one, 3-chloro-4-(4-chloro phenyl) -1-(2,4-dinitro phenylamino)azetidin-2-one, 3-chloro-1-(2,4-dinitrophenylamino)-4-(4-hydroxyphenyl) azetidin-2-one and 3-chloro-4-(2,4-dichloro phenyl)-1-(2,4-dinitro phenylamino) azetidin-2-one showed significant protection against carrageenan induced paw edema.

Keywords: Synthesis, azetidinones, nociception, inflammation

Introduction

Azetidin-2-one, a four-membered cyclic lactam (ß-lactam) skeleton has been recognized as a useful building block for the synthesis of a large number of organic molecules by exploiting the strain energy associated with it, in addition to its use in the synthesis of a variety of ß-lactam antibiotics. Efforts have been made in exploring such new aspects of ß-lactam chemistry versatile intermediates for the synthesis of aromatic ß-amino acids and their derivatives, peptides, polyamines, polyamino alcohols, amino sugars and polyamino ethers. The development of methodologies based on ß-lactam nucleus is now referred as 'the ß-lactam synthon methods'. The cyclic 2-azetidinone skeleton has been extensively used as a template to build the heterocyclic structure fused to the four-membered ring, using the chirality's and functionalisation of the ß-lactam nucleus as a stereo controlling element. This provides an access to diverse structural type of synthetic target molecules lacking ß-lactam ring structure. Azetidinones are reported for analgesic (Ishwar et al., 2003; Saturnino et al., 2000), anti-inflammatory (Gdupi et al., 1996; Srivastava et al., 1999; Srivastava et al., 2002), anti-bacterial (Ashok et al., 2003; Bhanvesh et al., 2004; Broccolo et al., 2006; Chavan et al., 2007; Choudhari et al., 2003; Davendra et al., 2002; Freddy et al., 2004; Girija et al., 2005;

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More et al., 2002; Oza et al., 2003; Padam et al., 2003; Patel et al., 2004; Pratibha et al., 2004), anti-convulsant (Srivastava et al., 1999; Srivastava et al., 2002), anti-cancer (Bimal et al., 2004; Veinberg et al., 2004), anti-tubercular (Kagthara et al., 2000) and vasopressin antagonist activities (Guillon et al., 2007). The biological effect of all azetidinones was mainly attributed due to the various 4-substituents on the azetidinone moiety. Based on this literature evidence, our research is focused on the incorporation of various 4-substituents on azetidinone moiety and hence some novel azetidinones were synthesized and evaluated for analgesic and anti-inflammatory activity.

Materials and Methods

Melting points were determined using a Veego melting point apparatus and are uncorrected. Thin layer chromatography was performed on a Merck Grade Silica gel GF_{254} plates of 0.25 mm thickness in chloroform: methanol (9:1) and ethyl acetate: methanol: ammonia (9:1:0.5). Spots were visualized using Iodine vapour. Infrared spectra were recorded on FTIR Spectrometer (Perkin Elmer, 1600 series) using potassium bromide discs. NMR spectra were recorded on Brucker 400 MHz NMR spectrophotometer. Chemical shifts are reported in parts per million (δ) units relative to internal standard tetramethylsilane. Elemental analysis was performed on Heraceus Carlo Erba 1108 and the analysis indicated that the elements were within ± 0.4 % of theoretical values. Synthetic scheme of azetidinone derivatives are shown in Figure 1.

Synthesis of N-benzylidene-N-{4-[(2,4-dintro-phenyl)-hydrazines (1a-i)

Six g (0.03 M) of 2,4-dinitro phenylhydrazine and various substituted aromatic aldehydes like benzaldehyde 3.18 g (0.03 M), 4-dimethyl amino benzaldehyde 4.47 g (0.03 M), 4-hydroxy-3-methoxy benzaldehyde 4.56 g (0.03 M), 4-chloro benzaldehyde 4.23 g (0.03 M), 4-hydroxy benzaldehyde 3.66 g (0.03 M), 3-nitro benzaldehyde 4.53 g (0.03 M), 4-methyl benzaldehyde 3.60 g (0.03 M), 2,4-dichloro benzaldehyde 5.25 g (0.03 M) and 2-methoxy benzaldehyde 4.08 g (0.03 M) were refluxed in presence of zinc-chloride 4.05 g (0.03 M) and methanol (50 mL) for 5 h at 100° C. After 5 h the reaction mixtures were filtered and the corresponding N-Benzylidene-N-{4-[(2,4-Dintro-phenyl)-hydrazines obtained were dried and recrystallized from ethanol. Melting point, percentage yield and other physical data of all the compounds **1a-i** were recorded in Tables 1 and 2.

Compound **1a:** IR: 3445 (N-H), 1606 (C=C), 1316, 1518 (NO_2), 1247 (C=N), 725 (aromatic hydrogen) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.5 (1H, d, J=7.1 Hz, CH-Ar), 7.4-8.1 (8H, m, Ar-H). Anal. Calcd for $C_{13}H_{10}N_4O_4$: C, 54.55; H, 3.52; N, 19.57; O, 22.36. Found: C, 54.18; H, 3.29; N, 19.48; O, 22.07.

Compound **1b:** IR: 3450 (N-H), 1606 (C=C), 1431(C-H), 1316, 1518 (NO_2), 1247 (C=N), 725 (aromatic hydrogen) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.5 (1H, d, J=7.1 Hz, CH-Ar), 1.8 (6H, s, $N(CH_3)_2$, 7.4-8.1 (7H, m, Ar-H). Anal. Calcd for $C_{15}H_{15}N_5O_4$: C, 54.71; H, 4.59; N, 21.27; O, 19.43. Found: C, 54.58; H, 4.34; N, 21.14; O, 19.28.

Compound **1c:** IR: 3583 (O-H), 3437 (N-H), 1596 (C=C), 1316, 1517 (NO_2), 1247 (C=N), 726 (aromatic hydrogen) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.5 (1H, d, J=7.1 Hz, CH-Ar), 2.2 (1H, s, OH), 2.8 (3H, s, OCH_3), 7.4-8.1 (6H, m, Ar-H). Anal. Calcd for $C_{14}H_{12}N_4O_6$: C, 50.61; H, 3.64; N, 16.86; O, 28.89. Found: C, 50.56; H, 3.52; N, 16.54; O, 28.74.

Compound **1d:** IR: 3438 (N-H), 1604, (C=C), 1320, 1508 (NO_2), 1247 (C=N), Cl (798), 727 (aromatic hydrogen) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.5 (1H, d, J=7.1 Hz, CH-Ar), 7.4-8.1 (7H, m, Ar-H). Anal. Calcd for $C_{13}H_9ClN_4O_4$: C, 48.69; H, 2.83; Cl, 11.06; N, 17.47; O, 19.96. Found: C, 48.56; H, 2.72; Cl, 11.02; N, 17.39; O, 19.86.

Compound **1e:** IR: 3590 (O-H), 3452 (N-H), 1614, 1598 (C=C), 1315, 1518 (NO_2), 1247 (C=N), 727 (aromatic hydrogen) cm^{-1}. . ^{1}H-NMR ($CDCl_3$) δ: 1.5 (1H, d, J=7.1 Hz, CH-Ar), 2.2 (1H, s, OH), 7.4-8.1 (7H, m, Ar-H). Anal. Calcd for $C_{13}H_{10}N_4O_5$: C, 51.66; H, 3.33; N, 18.54; O, 26.47. Found: C, 51.58; H, 3.29; N, 18.49; O, 26.38.

Table 1. Physical and analytical data of the synthesized compounds (**1a-i**)

Compound Number	R	Molecular Formula	Mol Wt	% Yield	Appearance	Color	M.P in °C	Rf * Value
1a	phenyl	$C_{13}H_{10}N_4O_4$	286.25	54	solid	dull white	98-103	0.49
1b	dimethyl aminophenyl	$C_{15}H_{15}N_5O_4$	329.32	56	solid	grey	123-129	0.72
1c	3-hydroxy-4-methoxy Phenyl	$C_{14}H_{12}N_4O_6$	333.28	78	solid	dull grey	189-194	0.57
1d	4-chloro Phenyl	$C_{13}H_9ClN_4O_4$	320.69	67	solid	pale yellow	156-163	0.62
1e	4-hydroxy phenyl	$C_{13}H_{10}N_4O_5$	302.24	59	solid	light green	177-183	0.81
1f	3-nitro Phenyl	$C_{13}H_9N_5O_6$	331.24	68	solid	yellow	166-171	0.78
1g	4-tolyl	$C_{14}H_{12}N_4O_4$	297.24	72	solid	light green	190-196	0.46
1h	2,4-dichloro phenyl	$C_{13}H_8Cl_2N_4O_4$	355.13	57	solid	white	157-163	0.66
1i	2-methoxy phenyl	$C_{14}H_{12}N_4O_5$	313.24	49	solid	dull white	189-195	0.56

*mobile phase used: ethyl acetate: methanol: ammonia (9:1:0.5)

Compound **1f:** IR: 3442 (N-H), 1619, 1596 (C=C), 1315, 1515 (NO_2), 1247 (C=N), 727 (aromatic hydrogen) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.5 (1H, d, J=7.1 Hz, CH-Ar), 7.4-8.1 (7H, m, Ar-H). Anal. Calcd for $C_{13}H_9N_5O_6$: C, 47.14; H, 2.74; N, 21.14; O, 28.98. Found: C, 47.06; H, 2.69; N, 21.09; O, 28.88.

Compound **1g:**. IR: 3438 (N-H), 1602, 1596 (C=C), 1430 (C-H), 1336, 1508 (NO_2), 1247 (C=N), 727 (aromatic hydrogen) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.5 (1H, d, J=7.1 Hz, CH-Ar), 2.35 (3H, s, Ar-CH_3), 7.4-8.1 (7H, m, Ar-H). Anal. Calcd for $C_{14}H_{12}N_4O_4$: C, 56.00; H, 4.03; N, 18.66; O, 21.31. Found: C, 56.06; H, 4.09; N, 18.58; O, 21.28.

Compound **1h:** IR: 3438 (N-H), 1610, 1594 (C=C), 1346, 1528 (NO_2), 1247 (C=N), Cl (798), 727 (aromatic hydrogen) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.5 (1H, d, J=7.1 Hz, CH-Ar), 7.4-8.1 (6 H, m, Ar-H). Anal. Calcd for $C_{13}H_8Cl_2N_4O_4$: C, 43.97; H, 2.27; Cl, 19.97; N, 15.78; O, 18.02. Found: C, 43.86; H, 2.19; Cl, 19.75; N, 15.70; O, 17.97.

Compound **1i:**. IR: 3440 (N-H), 1622, 1592 (C=C), 1336, 1508 (NO_2), 1247 (C=N), 727 (aromatic hydrogen) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.5 (1H, d, J=7.1 Hz, CH-Ar), 2.8 (3H, s, OCH_3), 7.4-8.1 (7H, m, Ar-H). Anal. Calcd for $C_{14}H_{12}N_4O_4$: C, 53.17; H, 3.82; N, 17.71; O, 25.29. Found: C, 53.05; H, 3.79; N, 17.69; O, 25.26.

Synthesis of 3-chloro-1-(2,4-dinitrophenylamino)-4-phenylazetidin-2-one (2a)

Ten g (0.1 M) of triethylamine and 11.3 g (0.1 M) chloroacetyl chloride were added drop wise at room temperature to 8.58 g (0.03 M) of **1a** in 1,4-dioxane (8.8 mL). The reaction mixture was stirred for 30 minutes and then refluxed for 3 h at 100° C. The solid mass obtained after the reaction were filtered and recrystallized from ethanol.

Compound **2a** was obtained in 97% yield as orange crystals. IR: 3438 (N-H), 1775 (C=O), 1619, 1596 (C=C), 1336, 1508 (NO_2), 1247 (C-N), Cl (800), 727 (aromatic hydrogen) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.5 (1H, d, J=7.1 Hz, CH-Ar), 3.1 (1H, d, J=7.1 Hz, CH-Cl), 7.4-8.1 (8H, m, Ar-H). Anal. Calcd for $C_{15}H_{11}ClN_4O_5$: C, 49.67; H, 3.06; Cl, 9.77; N, 15.45; O, 22.05. Found: C, 49.58; H, 3.09; Cl, 9.43; N, 15.34; O, 21.95.

Synthesis of 3-chloro-4-(4-(dimethylaminophenyl)-1-(2,4-dinitrophenylamino)-azetidin-2-one (2b)

Ten g (0.1 M) of triethylamine and 11.3 g (0.1 M) chloroacetyl chloride were added drop wise at room temperature to 9.87 g (0.03 M) of **1b** in 1,4-dioxane (8.8 mL). The reaction mixture was stirred for 30 minutes and then refluxed for 3 h at 100 ° C. The solid mass obtained after the reaction were filtered and recrystallized from ethanol.

Compound **2b** was obtained in 92% yield as brick red crystals. IR: 3438 (N-H), 1775 (C=O), 1619, 1596 (C=C), 1430 (C-H), 1336, 1508 (NO_2), 1247 (C-N), Cl (800), 727 (aromatic hydrogen) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.5 (1H, d, J=7.1 Hz, CH-Ar), 1.8 (6H, s, $N(CH_3)_2$), 3.1 (1H, d, J=7.1 Hz, CH-Cl), 7.4-8.1 (7 H, m, Ar-H). Anal. Calcd for $C_{17}H_{16}ClN_5O_5$: C, 50.32; H, 3.97; Cl, 8.74; N, 17.26; O, 19.71. Found: C, 50.14; H, 3.73; Cl, 8.34; N, 17.02; O, 19.41.

Synthesis of 3-chloro-1-(2,4-dinitrophenylamino)-4-(4-hydroxy-3-methoxyphenyl) azetidin -2-one (2c)

Ten g (0.1 M) of triethylamine and 11.3 g (0.1 M) chloroacetyl chloride were added drop wise at room temperature to 10 g (0.03 M) of **1c** in 1,4 dioxane (8.8 mL). The reaction mixture was stirred for 30 minutes and then refluxed for 3 h at 100 ° C. The solid mass obtained after the reaction were filtered and recrystallized from ethanol.

Compound **2c** was obtained in 86% yield as red crystals. IR: 3590 (O-H), 3438 (N-H), 2928 (C-H), 1775 (C=O), 1619, 1596 (C=C), 1336, 1508 (NO_2), 1247 (C-N), Cl (800), 727 (aromatic hydrogen) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.5 (1H, d, J=7.1 Hz, CH-Ar), 2.2 (1H, s, OH), 2.8 (3H, s, OCH_3), 3.1 (1H, d, J=7.1 Hz, CH-Cl), 7.4-8.1 (6 H, m, Ar-H). Anal. Calcd for $C_{16}H_{13}ClN_4O_7$: C, 47.01; H, 3.21; Cl, 8.67; N, 13.71; O, 27.40. Found: C, 46.81; H, 2.84; Cl, 8.43; N, 13.53; O, 27.20.

Synthesis of 3-chloro-4-(4-chlorophenyl)-1-(2,4-dinitrophenylamino)azetidin -2-one (2d)

Ten g (0.1 M) of triethylamine and 11.3 g (0.1 M) chloroacetyl chloride were added drop wise at room temperature to 9.62 g (0.03 mol) of **1d** in 1,4 dioxane (8.8 mL). The reaction mixture was stirred for 30 minutes and then refluxed for 3 h at 100 ° C. The solid mass obtained after the reaction were filtered and recrystallized from ethanol.

Compound **2d** was obtained in 75% yield as orange crystals. IR: 3438 (N-H), 1775 (C=O), 1619, 1596 (C=C), 1336, 1508 (NO_2), 1247 (C-N), Cl (800), 727 (aromatic hydrogen) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.5 (1H, d, J=7.1 Hz, CH-Ar), 3.1 (1H, d, J=7.1 Hz, CH-Cl), 7.4-8.1 (7 H, m, Ar-H). Anal. Calcd for $C_{15}H_{10}Cl_2N_4O_5$: C, 45.36; H, 2.54; Cl, 17.85; N, 14.11; O, 20.14. Found: C, 45.13; H, 2.43; Cl, 17.55; N, 14.21; O, 20.02.

Synthesis of 3-chloro-1-(2,4-dinitrophenylamino) -4-(4-hydroxyphenyl)azetidin -2-one (2e)

Ten g (0.1 M) of triethylamine and 11.3 g (0.1 M) chloroacetyl chloride were added drop wise at room temperature to 9 g (0.03 M) of **1e** in 1,4 dioxane (8.8 mL). The reaction mixture was stirred for 30 minutes and then refluxed for 3 h at 100 ° C. The solid mass obtained after the reaction were filtered and recrystallized from ethanol.

Compound **2e** was obtained in 79% yield as blackish brown crystals. IR: 3590 (O-H), 3438 (N-H), 1775 (C=O), 1619, 1596 (C=C), 1336, 1508 (NO_2), 1247 (C-N), Cl (800), 727 (aromatic hydrogen) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.5 (1H, d, J=7.1 Hz, CH-Ar), 2.2 (1H, s, OH), 3.1 (1H, d, J=7.1 Hz, CH-Cl), 7.4-8.1 (7 H, m, Ar-H). Anal. Calcd for $C_{15}H_{11}ClN_4O_6$: C, 47.57; H, 2.93; Cl, 9.36; N, 14.39; O, 25.35. Found: C, 46.43; H, 2.88; Cl, 9.06; N, 14.29; O, 25.04.

Synthesis of 3-chloro-1-(2,4-dinitrophenylamino) -4-(3-nitrophenyl)azetidin -2-one (2f)

Ten g (0.1 M) of triethylamine and 11.3 g (0.1 M) chloroacetyl chloride were added drop wise at room temperature to 10 g (0.03 M) of **1f** in 1,4 dioxane (8.8 mL). The reaction mixture was stirred

for 30 minutes and then refluxed for 3 h at 100 ° C. The solid mass obtained after the reaction were filtered and recrystallized from ethanol.

Compound **2f** was obtained in 74% yield as yellow crystals. IR: 3438 (N-H), 1775 (C=O), 1619, 1596 (C=C), 1336, 1508 (NO_2), 1247 (C-N), Cl (800), 727 (aromatic hydrogen) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.5 (1H, d, J=7.1 Hz, CH-Ar), 3.1 (1H, d, J=7.1 Hz, CH-Cl), 7.4-8.1 (7 H, m, Ar-H). Anal. Calcd for $C_{15}H_{10}ClN_5O_7$: C, 44.19; H, 2.47; Cl, 8.70; N, 17.08; O, 27.47. Found: C, 43.96; H, 2.62; Cl, 8.64; N, 17.08; O, 27.20.

Synthesis of 3-chloro-1-(2,4-dinitrophenylamino)-4-*p*-tolylazetidin-2-one (2g)

Ten g (0.1 M) of triethylamine and 11.3 g (0.1 M) chloroacetyl chloride were added drop wise at room temperature to 9 g (0.03 M) of **1g** in 1,4-dioxane (8.8 mL). The reaction mixture was stirred for 30 minutes and then refluxed for 3 h at 100 ° C. The solid mass obtained after the reaction were filtered and recrystallized from ethanol.

Compound **2g** was obtained in 93% yield as orange crystals. IR: 3438 (N-H), 2983 (C-H), 1775 (C=O), 1619, 1596 (C=C), 1430 (C-H), 1336, 1508 (NO_2), 1247 (C-N), Cl (800), 727 (aromatic hydrogen) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.5 (1H, d, J=7.1 Hz, CH-Ar), 2.35 (3H, s, Ar-CH_3), 3.1 (1H, d, J=7.1 Hz, CH-Cl), 7.4-8.1 (7 H, m, Ar-H). Anal. Calcd for $C_{16}H_{13}ClN_4O_5$: C, 51.01; H, 3.48;; Cl, 9.41; N, 14.87; O, 21.23. Found: C, 50.94; H, 3.29; Cl, 9.22; N, 14.78; O, 21.00.

Synthesis of 3-chloro-4-(2,4-chlorophenyl)-1-(2,4-dinitrophenylamino)azetidin -2-one (2h)

Ten g (0.1 M) of triethylamine and 11.3 g (0.1 M) chloroacetyl chloride were added drop wise at room temperature to 10.65 g (0.03 M) of **1h** in 1,4 dioxane (8.8 mL). The reaction mixture was stirred for 30 minutes and then refluxed for 3 h at 100 ° C. The solid mass obtained after the reaction were filtered and recrystallized from ethanol.

Compound **2h** was obtained in 86% yield as yellow crystals. IR: 3438 (N-H), 1775 (C=O), 1619, 1596 (C=C), 1336, 1508 (NO_2), 1247 (C-N), Cl (800), 727 (aromatic hydrogen) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.5 (1H, d, J=7.1 Hz, CH-Ar), 3.1 (1H, d, J=7.1 Hz, CH-Cl), 7.4-8.1 (6 H, m, Ar-H). Anal. Calcd for $C_{15}H_9Cl_3N_4O_5$: C, 41.74; H, 2.10; Cl, 24.64; N, 12.98; O, 18.53. Found: C, 41.65; H, 2.34; Cl, 24.46; N, 12.88; O, 18.25.

Synthesis of 3-chloro-1-(2,4-dinitrophenylamino)-4-(2-methoxy-3-methoxyphenyl) azetidin-2-one (2i)

Ten g (0.1 M) of triethylamine and 11.3 g (0.1 M) chloroacetyl chloride were added drop wise at room temperature to 9.39 g (0.03 M) of **1i** in 1,4 dioxane (8.8 mL). The reaction mixture was stirred for 30 minutes and then refluxed for 3 h at 100° C. The solid mass obtained after the reaction were filtered and recrystallized from ethanol.

Compound **2i** was obtained in 81% yield as reddish orange crystals. IR: 3438 (N-H), 1775 (C=O), 1619, 1596 (C=C), 1336, 1508 (NO_2), 1247 (C-N), Cl (800), 727 (aromatic hydrogen) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.5 (1H, d, J=7.1 Hz, CH-Ar), 2.8 (3H, s, OCH_3), 3.1 (1H, d, J=7.1 Hz, CH-Cl), 7.4-8.1 (7 H, m, Ar-H). Anal. Calcd for $C_{16}H_{13}ClN_4O_6$: C, 48.93; H, 3.34; Cl, 9.03; N, 14.27; O, 24.44. Found: C, 48.79; H, 3.01; Cl, 8.89; N, 14.01; O, 24.06.

Analgesic Activity

The analgesic activity was studied by Eddy's hot plate method (Eddy *et al.*, 1953). Albino mice of either sex weighing between 20 - 30 g were used for the study. The animals were housed and acclimated under standard laboratory conditions and were supplied with food and water *ad libitum.* All experiments were carried out with strict adherence to National Institutes of Health guide for the care and use of laboratory animals for pain experimentation, and were approved by the Institutional Animal Care Committee. The animals were divided into eleven groups, each consists of six animals. The first group received 0.5 % v/v Tween 80 (0.5 ml) suspension, the standard drug morphine (5 mg/kg) was administered intraperintoneally to the second group. The synthesized compounds (25 mg/kg in 0.5 % v/v Tween 80 suspension) **2a-i** were administered intraperintoneally to the remaining

groups respectively. The time of reaction to pain stimulus of the mice placed on the plate heated at 55° C ± 0.5 ° C was recorded before and after 15 minutes of the administration of the synthesized compounds. The increase in the reaction time against control group was recorded in Table 3.

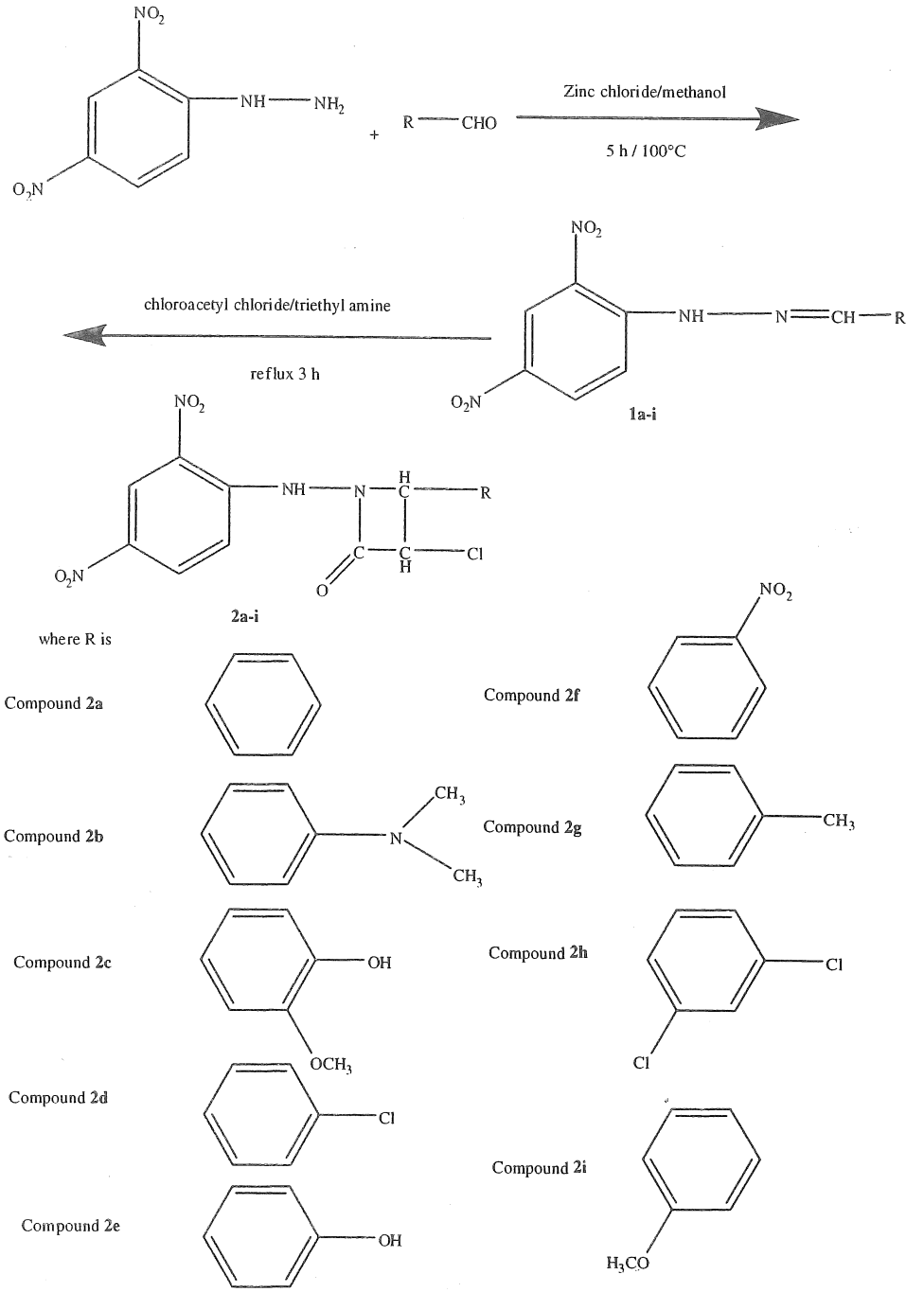

Fig. 1 Synthetic scheme of azetidinone derivatives

Table 2. Physical and analytical data of the titled compounds (**2a-i**)

IUPAC Name	No	R	Molecular Formula	Mol Wt	% Yield	Appearance	Color	M.P in °C	Rf Value
3-chloro-1-(2,4-dinitro phenylamino)-4-phenyl azetidin-2-one	**2a**	phenyl	$C_{15}H_{11}ClN_4O_5$	362.73	97	Solid crystal	Orange	160-165	0.49#
3-chloro-4-(4-(dimethylamino) phenyl)-1-(2,4-dinitrophenyl amino)azetidin-2-one	**2b**	dimethyl aminophenyl	$C_{17}H_{16}ClN_5O_5$	405.79	92	Solid crystal	Brick red	220-228	0.73*
3-chloro-1-(2,4-dinitrophenylamino)-4-(3-hydroxy-4-methoxyphenyl)azetidin-2-one	**2c**	3-hydroxy-4-methoxy phenyl	$C_{16}H_{13}ClN_4O_7$	408.75	86	Solid crystal	Red	220-221	0.59#
3-chloro-4-(4-chlorophenyl)-1-(2,4-dinitrophenylamino)azetidin-2-one	**2d**	4-chloro phenyl	$C_{15}H_{10}Cl_2N_4O_5$	397.17	75	Amorphous Powder	Orange	205-210	0.62*
3-chloro-1-(2,4-dinitrophenylamino)-4-(4-hydroxyphenyl)azetidin-2-one.	**2e**	4-hydroxy phenyl	$C_{15}H_{11}ClN_4O_6$	378.73	79	Solid crystal	Blackish brown	210-215	0.69#
3-chloro-1-(2,4-dinitrophenylamino)-4-(3-nitrophenyl)azetidin-2-one.	**2f**	3-nitro phenyl	$C_{15}H_{10}ClN_5O_7$	407.72	74	Solid crystal	Yellow	197-200	0.54#
3-chloro-1-(2,4-dinitrophenylamino)-4-p-tolylazetidin-2-one.	**2g**	4-tolyl	$C_{16}H_{13}ClN_4O_5$	376.75	93	Solid crystal	Orange	200-205	0.45*
3-chloro-4-(2,4-dichlorophenyl)-1-(2,4-dinitrophenylamino)azetidin-2-one.	**2h**	2,4-dichloro phenyl	$C_{15}H_9Cl_3N_4O_5$	431.62	86	Solid crystal	Yellow	200-202	0.46*
3-chloro-1-(2,4-dinitrophenylamino)-4-(2-methoxypheny)azetidin-2-one.	**2i**	2-methoxy phenyl	$C_{16}H_{13}ClN_4O_6$	392.76	81	Solid crystal	Reddish orange	220-223	0.57#

* mobile phase used: chloroform: methanol (9:1)
\# ethyl acetate: methanol: ammonia (9:1:0.5)

Anti-inflammatory activity

The anti-inflammatory activity was evaluated by carrageenan induced paw edema method (Winter *et al.*, 1962). Albino rats of Wistar strain weighing 100-200 g of either sex were divided into eleven groups each of six animals. The animals were maintained under normal environmental conditions. They were fed *ad libitum* with standard feed and water. Tween 80 suspension (0.5 % v/v) of the test compounds were administered intraperitoneally in a dose of 25 mg kg^{-1}. The control group was given only 0.5 % v/v Tween 80 (0.5 ml) suspension. One group was administered with diclofenac sodium as standard, intraperitoneally in a dose of 2 mg kg^{-1}. After 30 minutes of the administration of test compounds and diclofenac sodium paw edema was induced in albino rats by injecting 0.1 mL of carrageenan (1% v/v in normal saline) suspension, into subplantar region of the left hind paw of each rat. After 3 h of carrageenan injection, the increase in paw volume was measured by a plethysmometer (Inco, India). The anti-inflammatory activity was measured in terms of percentage inhibition of edema and is analyzed statistically by students "t" test and recorded in Table 4.

Table 3. Analgesic activity of the titled compounds (**2a-i**)

Treatment	Reaction Time Sec (Mean ± SEM)	
	Before drug administration	After drug administration
Control	4.2 ± 0.24	4.3 ± 0.18
Morphine	4.4 ± 0.01**	14.0 ± 0.09**
2a	3.2 ± 0.24**	5.4 ± 0.02**
2b	4.6 ± 0.07*	4.8 ± 0.05**
2c	4.8 ± 0.18	9.8 ± 0.09**
2d	3.5 ± 0.07**	3.6 ± 0.06**
2e	4.6 ± 0.08**	8.2 ± 0.13**
2f	4.5 ± 0.07*	5.6 ± 0.11**
2g	4.3 ± 0.07	6.4 ± 0.28**
2h	4.4 ± 0.06	12.4 ± 0.07**
2i	3.8 ± 0.10	4.5 ± 0.06

**p < 0.001 vs control is highly significant
* p < 0.01 vs control is significant

Table 4. Anti-inflammatory activity of the titled compounds (**2a-i**)

Compound	Paw volume (mean ± SEM)	Percentage Inhibition after 3 h
Control	0.53 ± 0.156	------
Diclofenac sodium	0.14 ± 0.023	73.58*
	0.38 ± 0.028	28.30
2a	0.32 ± 0.045	39.62
2b	0.21 ± 0.038	60.37*
2c	0.20 ± 0.034	62.22*
2d	0.19 ± 0.124	64.15
2e	0.33 ± 0.030	37.73
2f	0.30 ± 0.040	43.39
2g	0.20 ± 0.220	62.22*
2h	0.35 ± 0.124	33.96
2i		

*p < 0.001 vs control is significant

Results and Discussion

Analgesic activity

Eddy's hot plate method was adopted for the evaluation of analgesic activity of the synthesized compounds, because it is most widely used method for evaluating central analgesic activity of the NCE's. In this method heat is used as source of pain. The prolongation of the latency times comparing the values before and after administration of the test compounds indicates the presence of analgesic activity. Compounds **2c**, 3-chloro-1-(2,4-dinitrophenylamino)-4-(3-hydroxy-4-methoxyphenyl)azetidin-2-one, **2e**, 3-chloro-1-(2,4-dinitrophenylamino)-4-(4-hydroxyphenyl)azetidin-2-one and **2h** 3-chloro-4-(2,4-dichloro phenyl)-1-(2,4-dinitro phenylamino)azetidin-2-one showed significant analgesic activity by increasing the reaction time. Compound **2h** with 2,4-dichlorophenyl substitution of azetidinone showed promising analgesic activity compared to other compounds. Compounds **2a**, 3-chloro-1-(2,4-dinitro phenylamino)-4-phenyl azetidin-2-one, **2f**, 3-chloro-1-(2,4-dinitro phenylamino)-4-(3-nitrophenyl)azetidin-2-one and **2g**, 3-chloro-1-(2,4-dinitro phenylamino)-4-p-tolyl azetidin-2-one showed moderate analgesic activity. Compounds **2b**, 3-chloro-4-(4-(dimethylamino)phenyl)-1-(2,4-dinitrophenyl amino)azetidin-2-one, **2d**, 3-chloro-4-(4-chlorophenyl)-1-(2,4-dinitrophenylamino)azetidin-2-one and **2i**, 3-chloro-1-(2,4-dinitro phenylamino)-4-(2-methoxy phenyl)azetidin-2-one were devoid of analgesic activity.

Anti-inflammatory activity

Inflammatory edema induced by carrageenan provides a rapid and sensitive means for evaluating anti-inflammatory agents. Among the compounds tested compound **2e**, 3-chloro-1-(2,4-dinitrophenylamino)-4-(4-hydroxyphenyl)azetidin-2-one showed promising anti-inflammatory activity. Compounds **2c** 3-chloro-1-(2,4-dinitrophenylamino)-4-(3-hydroxy-4-methoxyphenyl)azetidin-2-one, **2d**, 3-chloro-4-(4-chlorophenyl)-1-(2,4-dinitrophenylamino) azetidin-2-one and **2h**, 3-chloro-4-(2,4-dichloro phenyl)-1-(2,4-dinitro phenylamino) azetidin-2-one showed significant protection against carrageenan induced paw edema.. Compound **2d** and **2h** with chlorophenyl and dichlorophenyl substitution at azetidinone ring exhibited same anti-inflammatory activity. Compound **2f**, 3-chloro-1-(2,4-dinitro phenylamino)-4-(3-nitrophenyl)azetidin-2-one, **2g** 3-chloro-1-(2,4-dinitro phenylamino)-4-p-tolyl azetidin-2-one and **2i**, 3-chloro-1-(2,4-dinitro phenylamino)-4-(2-methoxy phenyl)azetidin-2-one showed moderate protection against carrageenan induced paw edema.

References

Ashok, K., Pratibha, S., Ravi, S. and Pankaj, M. (2003).Synthesis and anti-microbial screening of N Substituted-3-chloro-4-dithiocarbamate azetidin-2-ones. *Indian J. Chem.* 42B:416-420.

Bhanvesh, D.N. and Desai, K.R.(2004).Synthesis of some heterocyclic Schiff base and azetidinone compounds and their anti-bacterial activity. *Asian J. Chem.* 16:1749-1752.

Bimal, K.B., Frederick, F.B. and Indrani, B. (2004). Synthesis of anticancer β-lactams: mechanism of action. *Bioorganic Med. Chem.* 12:2523-2528.

Broccolo, F., Cainelli, G. and Caltabiano, G. (2006). Design, synthesis, and biological evaluation of 4-alkyliden-beta lactams: new products with promising antibiotic activity against resistant bacteria. *J Med. Chem. 49:2804-2811.*

Chavan, A.A. and Pai, N.R. (2007). Synthesis and Biological Activity of N-Substituted-3-chloro-2-azetidinones. *Molecules* 12: 2467-2477.

Choudhari, B.P. and Mulwad, V.V. (2003). Synthesis and antimicrobial screening of N- [6'-coumarinyl amino-3-chloro-4-aryl azetidine-2-ones. *Indian J. Heterocycl. Chem.* 12:197-200.

Devendra, K. and Sharma, R.C.(2002).Synthesis and antimicrobial profile of 5-imidazolinones, Sulphonamides, azemethine, 2-azetidinones and formazans derived from 2-amine, 3-cyano- 5(5'-

chloro-3-methyl-1-phenyl pyrazol-4'-yl vinyl)7,7'-dimethyl-6,7-dihydro benzo(b)thiophenes. *J Indian Chem. Soc.* 79: 284-285.

Eddy, N.B. and Leimbach, D.J. (1953). Synthetic analgesic (II): dithienylbutenyl and dithienylbutylamines. *J. Pharmcol. Exp. Ther.* 107:385-396.

Freddy, H. and Sushil Kumar, M.(2004).Synthesis of some azetidin-2-ones and thiazolidin-4-ones as potential antimicrobial agents. *Indian J. Heterocycl. Chem.* 13:197-200.

Gdupi, R. H. Jeeson, M. and Bhat, A . (1996). Synthesis of 2(2'-carboxy-5'-nitro phenyl) 3,4-substituted azetidin-2-ones as anti-inflammatory and anti-microbial agents. *Indian J. Heterocycl. Chem.* 6:196-199.

Girija, S.S. and Boycie, J.M. (2005). Synthesis of 2-azetidinones from 2-diazo-1, 2-diarylethanones and N-(2-thienylidene)imines as possible antimicrobial agents. *Il Farmaco* 60:727-730.

Guillon, C.D., Koppel, G.A., Brownstein, M.J., Chaney, M.O., Ferris, C.F., Lu, S.F., Fabio, K.M., Miller, M. J., Heindel, N. D. and Hunden, D.C. (2007). Azetidinones as vasopressin V1a antagonists. *Bioorganic Med. Chem.* 15:2054-2080.

Ishwar, B.K., Mubeen, M. and Kalluraya, B. (2003). Synthesis and pharmacological studies of some azetidinones bearing Ibuprofen moiety. *Indian J. Heterocycl. Chem.* 13:183-184.

Kagthara, P., Teja, S., Rajeev, D. and Parekh, H. H. (2000). Synthesis of some 2-azetidinones as potential antitubercular agents. *Indian J. Heterocycl. Chem.* 10:09-12.

More, S.V., Dongarkhadekar, D.V., Charan, R.N., Jadhav, W.N. and Bhusare, P. (2002). Synthesis and antibacterial activity of new Schiff base 4- thiazolidones and 2-azetidinones. *J. Indian Chem. Soc.* 79:768-769.

Oza, H.B., Datta, N.J., Joshi, D.G., Parekh, H.H. (2003). Synthesis of some New-2-azetidinones as potential antimicrobial agents, *Indian J. Heterocycl. Chem.* 3:275-276.

Padam, K. and Saksena, R.K. (2003). Synthesis and antimicrobial activity of some new 2-phenyl-3-p(2'-methyl-3'-aryl-4'-oxo thiazolin-2'yl)phenyl quinazolin-4-ones and 2-phenyl-3- p(1'-ayrl-3-phthalimido-4'-methyl azetidin-2'-one-2'yl)phenyl quinazolin-4-ones. *Indian J. Heterocycl. Chem.* 12:315-318.

Patel, H.S. and Patel, V.K. (2004). Synthesis and anti-microbial activity of some new N(4-4' disubstituted amino sulphonyl phenyl) -3-chloro-4-phenyl azetidine-2-ones. *Oriental J. Chem.* 17:425-428.

Pratibha, S., Ashok, K. and Shikha, S. (2004). Bisheterocyclic synthesis and anti-microbial studies of some biologically significant 2(N-3-chloro-4-substituted azetidinone-2)amino-4-hydroxy purines. *Indian J. Chem.* 43B:385-388.

Saturnino, C., Fusco, B., Saturnino, P., De Martino, G., Rocco, F. and Lancelot, J.C. (2000). Evaluation of analgesic and anti-inflammatory activity of novel beta-lactam monocyclic Compounds. *Biol. Pharm. Bull.* 23:654-656.

Srivastava, S.K., Srivastava, S. and Srivastava, S.D. (1999). Synthesis of new carbazolyl thiadiazolyl-2-oxoazetidines as antimicrobial, anticonvulsant and anti-inflammatory agents. *Indian J. Chem.* 38B:183-187.

Srivastava, S.K. and Srivastava, S.(2002).Synthesis of New 1,2,4-triazolo thiadiazoles and 2-oxoazetidines as anti-microbial, anti-convulsant and anti-inflammatory agents. *Indian J. Chem.* 41(B): 2357-2363.

Veinberg, S. and Vorona, K. (2004). Synthesis of antitumor 6-alkylidene penicillamate sulfones and related 3-alkylidene-2-azetidinone. *Bioorganic Med. Chem.* 12:147-150.

Winter, C.A, Risley, E. A. and Nuss, G. W. (1962). Carrageenan-induced edema in hind paws of the rats as an assay for anti-inflammatory drugs. *Exp. Biol. Med.* 111: 544-547

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