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Synthesis and characterization of some novel azetidinone derivatives as anti-bacterial and anti-convulsant agents

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Abstract
Several novel azetedinones have been synthesized by condensation of 2, 4-dinitro phenyl hydrazine with various substituted aromatic aldehydes in presence of zinc chloride and methanol followed by the reaction with chloroacetyl chloride and triethylamine. The synthesized compounds were characterized by IR and PMR analysis. The titled compounds were evaluated for anti-bacterial and anticonvulsant activity by cup plate method and maximal electroshock induced convulsion method respectively. Compounds 3-chloro-1-(2,4-dinitrophenylamino)-4-(3-hydroxy-4-methoxyphenyl)azetidin-2-one **2c**, 3-chloro-4-(4-chlorophenyl)-1-(2,4-dinitrophenyl amino)azetidin-2-one **2d**, 3-chloro-1-(2,4-dinitrophenylamino)-4-(3-nitrophenyl)azetidin-2-one **2f** and 3-Chloro-1-(2,4-dinitrophenylamino)- 4-*p*-tolyl-azetidin-2-one **2g** showed good anti-bacterial activity. Compounds 3-chloro-1-(2,4-dinitro phenylamino)-4-(3-nitrophenyl)azetidin-2-one **2f** and 3-chloro-4-(2,4-dichloro phenyl)-1-(2,4-dinitro phenylamino)azetidin-2-one **2h** elicited good anti-convulsant activity when compared with control. Compound 3-chloro-1-(2,4-dinitrophenylamino)-4-(4-hydroxyphenyl)azetidin-2-one **2e** elicited moderate anti-convulsant activity when compared with control.

Keywords: Azetidinone derivatives; anti-bacterial activity; anti-convulsant activity.

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 and functionalisation of the ß-lactam nucleus as a stereo controlling element. This provides an access to diverse structural type of synthetic target molecules

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lacking ß-lactam ring structure. Azetidinones are reported for analgesic (Ishwar Bhat *et al.*, 2003) (Saturnino *et al.*, 2000), anti-inflammatory (Srivastava *et al.*, 2002), (Srivastava *et al.*, 1999), (Gdupi *et al.*, 1996), anti-bacterial (Singh and Mmolotsi, 2005) (Bhanvesh and Desai, 2004), (Patel and Patel, 2004), (Pratibha *et al.*, 2004), (Ashok *et al.*, 2003), (Devendra and Sharma, 2002), (More *et al.*, 2002), (Choudhari and Mulwad, 2003), (Oza *et al.*, 2003), (Padam and Saksena, 2003), (Freddy and Sushil Kumar, 2004), anti-convulsant (Srivastava *et al.*, 2002), (Srivastava *et al.*, 1999), (Singh *et al.*, 1994), anti-cancer (Veinberg and Vorona, 2004) and anti-tubercular (Kagthara *et al.*, 2000) activities. Hydrogen at C4 position of the azetidinone ring is highly active. Based on these facts, some novel azetidinones were synthesized and evaluated for antibacterial and anticonvulsant activity.

Experimental

Melting points were determined in Veego melting point apparatus and are not corrected. Thin layer chromatography was performed on a Merck Grade Aluminum foil GF_{254} plates of 0.25 mm thickness in chloroform: Methanol system (9:1). Spots were visualized using Iodine vapor. Infrared spectra were recorded on Perkin Elmer 1600 series FTIR Spectrometer using potassium bromide discs. PMR 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.

Synthesis of N-Benzylidene-N-{4-[(2,4-Dintro-phenyl)-hydrazines (1a-i). Equimolar quantities of 2,4-dinitro phenyl hydrazine and various substituted aromatic aldehydes like benzaldehyde, 4-dimethyl amino benzaldehyde, 4-hydroxy-3-methoxy benzaldehyde, 4-chloro benzaldehyde, 4-hydroxy benzaldehyde, 3-nitro benzaldehyde, 4-methyl benzaldehyde, 2,4-dichloro benzaldehyde and 2-methoxy benzaldehyde were refluxed in the presence of zinc-chloride and methanol 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.

Synthesis of derivatives of azetidin-2-ones (2a-i). Equimolar quantities of triethylamine and chloroacetyl chloride were added drop wise at room temperature to the solution of N-Benzylidene-N-{4-[(2,4-Dintro-phenyl)-hydrazines in 1,4-dioxan. 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.

All the nine synthesized compounds showed strong absorption bands in infra red region (KBr, 9 value in cm^{-1}) for NO_2 1315 and 1515, N-H 3300, C-N 1360, C=O 1775, Cl 800 and for aromatic absorptions at 3100, 1500, 770. In addition to the above, compounds **2b, 2g** showed absorption bands for CH_3 at 1430, compound **2c** and **2e** for OH at 3590.

All the nine synthesized compounds showed PMR signals (in $CDCl_3$) as a doublet at δ 1.5 for the CH proton adjacent to aryl group, a doublet at δ 3.1 for CH proton adjacent to chlorine, multiplet from δ 7.4-8.1 for aromatic proton. The NH proton could not be detected. In addition to the above signals compound **2b** showed a singlet signal for N-CH_3 proton at δ 1.4, compound **2c** showed a broad singlet signal for OH proton at δ 2.2 and a singlet signal for OCH_3 at 1.3, compound **2e** showed a singlet signal for OH proton at δ 2.2, compound **2g** showed a singlet for CH_3 protons at δ 2.3 and compound **2i** showed a singlet for OCH_3 at δ 2.5.

Anti-bacterial activity. Antibacterial activity was evaluated by agar cup plate method (Mukerjee, 1996). Nutrient agar medium was prepared by dissolving peptone (10 g), beef extract (10 g) and sodium chloride in purified water (1000 ml) and the pH of the media was adjusted to 8.4 with 5M sodium hydroxide solution. To this solution agar (20 g) was added , boiled and stirred thoroughly until the agar was dissolved. Then 20 ml of this nutrient agar medium was transferred into each boiling tube and plugged with non-absorbent cotton. The tubes containing the nutrient agar medium were sterilized by pressure controlled heat sterilization technique using an autoclave at 15 lbs and 115°C for 20 minutes. After sterilization the nutrient agar medium was melted, cooled and inoculated with three Gram positive organism *Bacillus subtilis* (*Bacillus subtilis* NCIM 2063), *Shigella niger* (*Shigella niger* NCIM 2079), *Proteus vulgaris* (*Proteus vulgaris NCIM 2027)* and one Gram negative organism *Escherichia coli (Escherichia coli NCIM 2065)* and poured into sterile Petri dish to get a uniform thickness of 6 mm. Cups were made out in the other plate using sterile cork borer (6 mm diameter). The standard antibacterial agent ciprofloxacin (250 µg/ml) and solvent control (2 % v/v Tween 80) and the synthesized compounds in a concentration of 250 µg/ml were added with the sterile micro pipette into each cup. The plates were kept in the refrigerator for 6 hours and then incubated at 37°C for 24 hours and the diameter of zone of inhibition were measured and recorded in Table 2.

Anti-convulsant activity. The anti-convulsant activity was evaluated by maximal electroshock induced seizure (MES) method (Kulkarni, 1999). Swiss albino mice weighing 25-30 g of either sex were divided into ten groups each of six animals. The control group of animals was administered with 0.5%v/v Tween 80 (0.5) ml suspension. One group was administered with phenobarbitone (Nicholas Primal) as standard, intraperitoneally in a dose of 20 mg/kg. Tween 80 suspensions (0.5 % v/v) of the test compounds were administered intraperitoneally in a dose of 20 mg/kg for other group of animals. After 1 hour of the administration of the test compounds and standard, the animals were given electroshock through ear electrodes of 150 mA for 0.2 sec by electroconvulsiometer. Onset times for tonic, flexion, extension and clonic phase were noted. The protective index was observed as reduction time of tonic extensor phase and was tabulated in Table 3.

Results and Discussion

Anti-bacterial activity. The titled compounds 3-chloro-1-(2,4-dinitrophenylamino)-4-(3-hydroxy-4-methoxyphenyl) azetidin-2-one **2c** showed zone of inhibition against *Bacillus subtilis.* 3-chloro-4-(4-chlorophenyl)-1-(2,4-dinitrophenylamino)azetidin-2-one **2d** and 3-chloro-1-(2,4-dinitrophenylamino)-4-p-tolyl azetidin-2-one **2g** showed zone of inhibition against *Shigella niger.* 3-chloro-1-(2,4-dinitrophenykamino)-4-(3-nitrophenyl) azetidin-2-one **2f** showed zone of inhibition against *E. coli.* The other compounds **2a, 2b, 2e, 2h** and **2i** did not show any zone of inhibition against the tested microorganism and hence they are devoid of anti-bacterial activity. The standard drug Ciprofloxacin showed zone of inhibition against the entire tested microorganism.

Anti-convulsant activity. MES method was used for the evaluation of anti-convulsant activity, because it is the most widely employed seizure models for the early identification and high throughput screening of investigational anti-convulsant drugs. Compounds 3-chloro-1-(2,4-dinitro phenyl amino)-4-(3-nitrophenyl)azetidin-2-one **2f** and 3-chloro-4-(2,4-dichloro phenyl)-1-(2,4-dinitro phenylamino) azetidin-2-one **2h** reduced markedly the duration of extensor phase when compared to control and hence it

was concluded that compounds **2f** and **2h** possess good anti-convulsant activity. Compound 3-chloro-1-(2,4-dinitrophenyl amino)-4-(4-hydroxyphenyl) azetidin-2-one **2e** showed moderate anticonvulsant activity. The standard drug phenobarbitone at a dose of 20 mg/kg produced drastic reduction in the duration of extensor phase. The compounds 3-chloro-1-(2,4-dinitro phenylamino)-4-phenyl azetidin-2-one **2a**, 3-chloro-4-(4-(dimethyl amino) phenyl)-1-(2,4-dinitrophenyl amino) azetidin-2-one **2b**, 3-chloro-1-(2,4-dinitro phenylamino)-4-p-tolyl azetidin-2-one **2g** and 3-chloro-1-(2,4-dinitro phenylamino)-4-(2-methoxy phenyl)azetidin-2-one **2i** elicited mild anti-convulsant activity. The other compound 3-chloro-1-(2,4-dinitrophenyl amino)-4-(3-hydroxy-4-methoxyphenyl) azetidin-2-one **2c** showed no anti-convulsant activity.

Table 1: Physical and analytical data of the titled compounds

IUPAC Name	No	R	Molecular Formula	Mol. Wt.	% Yield	Appearance	Color	m.p. (°C)	R_f value
3-chloro-1-(2,4-dinitro phenylamino)-4-phenyl azetidin-2-one	**2a**	Phenyl dimethyl	$C_{15}H_{11}N_4O_5Cl$	362.73	97	Solid crystal	Orange	160-165	0.75
3-chloro-4-(4-(dimethylamino) phenyl)-1-(2,4-dinitro phenylamino) azetidin-2-one	**2b**	Aminophenyl	$C_{17}H_{16}N_5O_5Cl$	405.79	92	Solid crystal	Brick red	220-228	0.92
3-chloro-1-(2,4-dinitrophenylamino)-4-(3-hydroxy-4-methoxyphenyl)azetidin-2-one	**2c**	3-ydroxy-4-methoxy Phenyl	$C_{16}H_{13}N_4O_7Cl$	408.75	86	Solid crystal	Red	220-221	0.75
3-chloro-4-(4-chloro phenyl)-1-(2,4-dinitro phenylamino)azetidin-2-one	**2d**	4-chloro phenyl	$C_{15}H_{10}N_4O_5Cl_2$	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	$C_{15}H_{11}N_4O_6Cl$	378.73	79	Solid crystal	Blackish brown	210-215	0.75
3-chloro-1-(2,4-dinitrophenylamino)-4-(3-nitrophenyl) azetidin-2-one	**2f**	3-nitro Phenyl	$C_{15}H_{10}N_5O_7Cl$	407.72	74	Solid crystal	Yellow	197-200	0.75
3-chloro-1-(2,4-dinitrophenylamino)-4-p-tolylazetidin-2-one	**2g**	4-tolyl	$C_{16}H_{13}N_4O_5Cl$	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	$C_{15}H_9N_4O_5Cl_3$	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	$C_{16}H_{13}N_4O_6Cl$	392.76	81	Solid crystal	Reddish orange	220-223	0.75

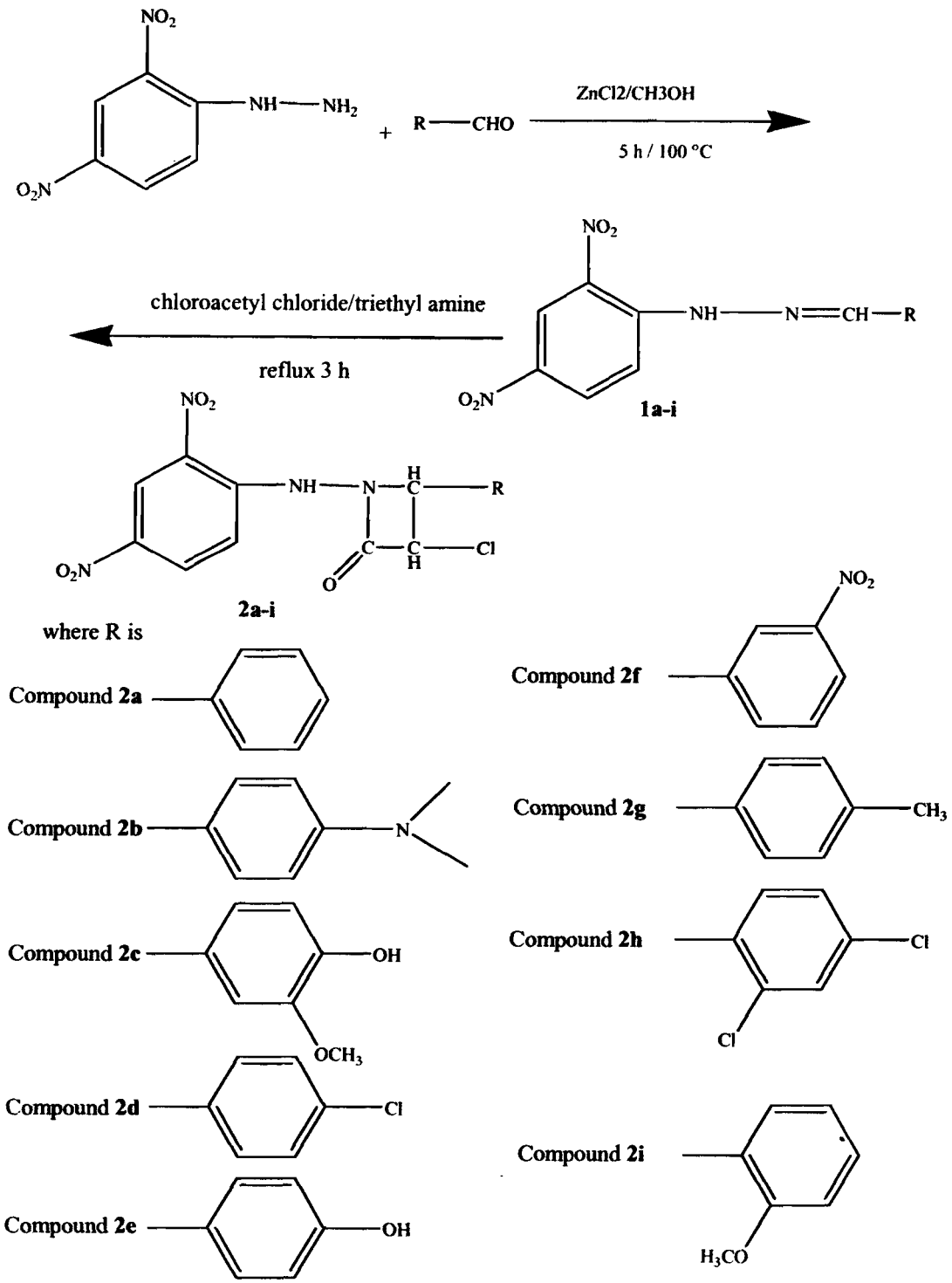

Fig. 1. Synthetic scheme of Azetidinone derivatives

Table 2: Antibacterial activity data of the synthesized compounds

Compound No.	Zone of inhibition in mm			
	B. subtilis	*S. niger*	*P. vulgaris*	*E. coli*
2a	00	00	00	00
2b	00	00	00	00
2c	14	00	00	00
2d	00	16	00	00
2e	00	00	00	00
2f	00	00	00	19
2g	00	09	00	00
2h	00	00	00	00
2i	00	00	00	00
Ciprofloxacin	20	18	19	23

Table 3: Anti-convulsant activity data of the synthesized compounds

S/No	Dose (mg/kg)	Compound	Duration (Mean ± SEM, sec)			Recovery/ Death
			Extensor	Clonus	Stupor	
1	-	Control	25.5±0.37	30±0.51	60.1±0.29	Recovery
2	20	Phenobarbitone	8.2±0.37**	5.0±0.51**	5.5±0.29**	Recovery
3	20	**2a**	15.5±0.43**	5.1±0.46**	20.1±0.31**	Recovery
4	20	**2b**	15.5±0.46**	10.1±0.46**	10.0±0.28**	Recovery
5	20	**2c**	21.2±0.52**	26.0±0.44**	40.1±0.31**	Recovery
6	20	**2d**	16.6±0.58**	8.1±0.46**	15.1±0.31**	Recovery
7	20	**2e**	14.5±0.43**	18.1±0.46**	27.1±0.31**	Recovery
8	20	**2f**	13.0±0.39**	16.1±0.46**	28.0±0.28**	Recovery
9	20	**2g**	16.6±0.43**	20.6±0.57**	25.6±0.32**	Recovery
10	20	**2h**	12.5±0.43**	18.5±0.51**	25.8±0.31**	Recovery
11	20	**2i**	17.5±0.43**	12.1±0.46**	35.8±0.31**	Recovery

**$p < 0.001$ vs. Control highly significant

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