method was rugged as percentage recovery was found to in the range of 99-101%. The robustness of the method was determined by making slight changes in the chromatographic conditions. Buffer pH modification did not have any significant effect. The effect of organic strength on retention time was studied by small change in percentage polarity of mobile phase system and it was found that even slight percentage change in volume or concentration of acetonitrile in the ratio changed the retention time. The system suitability tests were carried out as per USP XXIV requirements. System suitability tests were carried out on freshly prepared standard stock solution of metoprolol tartrate and hydrochlorthiazide and the parameters obtained with 20 µl injection volume. The number of theoretical plates for hydrochlorthiazide and metoprolol tartrate was calculated as 6404 and 9464, respectively. The symmetry factor for hydrochlorthiazide and metoprolol tartrate was 1.25 and 1.17, respectively. The resolution between the two peaks was 2.9. The obtained results confirmed that the method is highly suitable for its intended purpose of separation of metoprolol tartrate and hydrochlorthiazide and its simultaneous determination in formulation.

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Synthesis and Biological Evaluation of 4-(Substituted Aryl)-1-(N-Indolyl Acetamidyl)-3-Chloro-2-Azetidinones

P. V. R. CHOWDARY*, S. G. JAWARKAR, K. SHASHIKALA RANI, GIRIJA RAO[1] AND V. PRASAD[1]

Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, MAHE, Manipal-576 104

[1]Department of Microbiology, Kasturba Medical College, MAHE, Manipal-576 104

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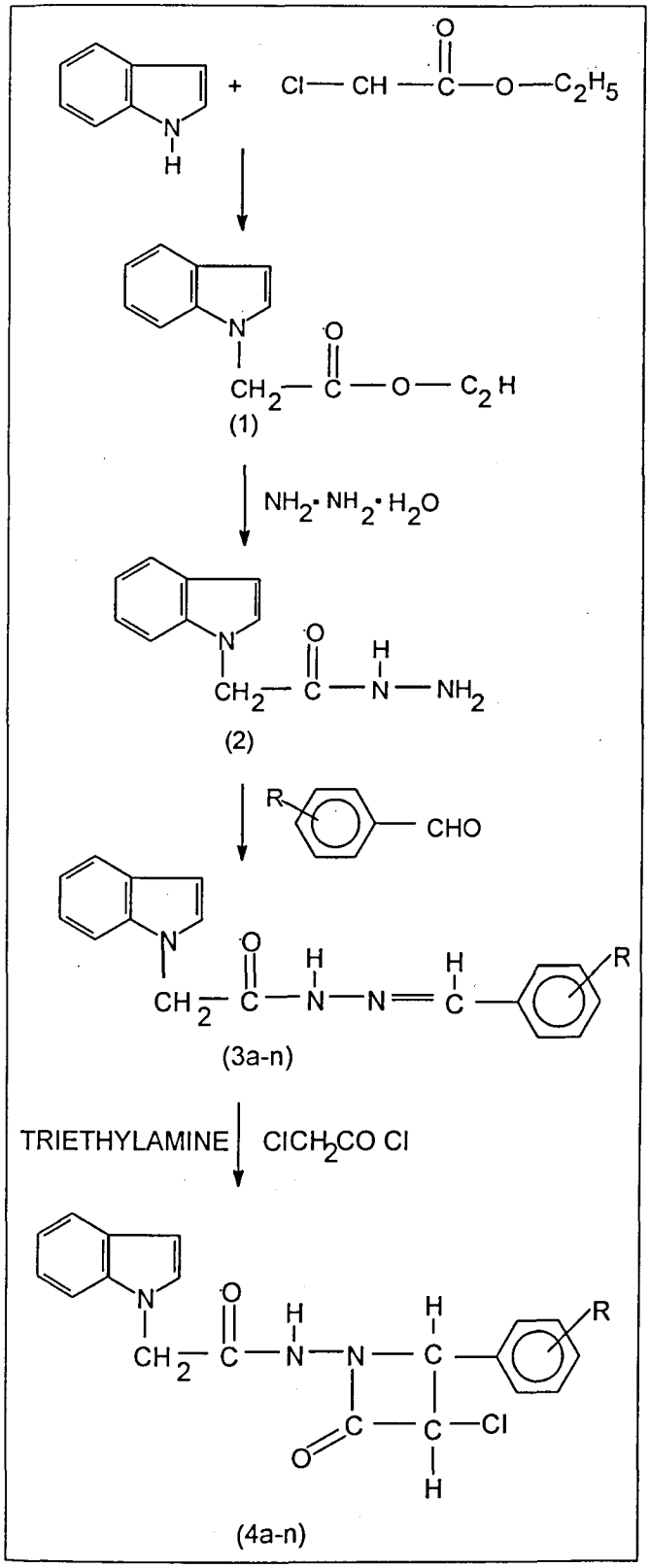
Various 4-(substituted aryl)-1-(N-indolyl acetamidyl)-3-chloro-2-azetidinones were prepared by condensing N-(arylidine hydrazidomethyl)-indoles with chloroacetyl chloride. Their structures were established by chemical tests, IR and ^{1}HNMR spectral data. These synthesized compounds were tested for their antibacterial, antifungal, antitubercular and antiinflammatory activities.

In continuation of our studies on indoles[1], we report herein the synthesis and biological evaluation of 4-(substituted aryl)-1-(N-indolyl acetamidyl)-3-chloro-2-azetidinones. 2-Azetidinones especially with substituents at 1,4 positions have been found to possess significant antibacterial, antifungal, antitubercular and antiinflammatory activities[2-7]. The incorporation of 4-(substituted aryl)-2-azetidinones moiety to indole framework was thought to enhance the biological activities because the results were quite encouraging when different functionalities were in-

***For correspondence**

E-mail: pvrchowdary2003@yahoo.co.in



Scheme 1: Synthesis of 2-azetidinones

troduced in the 2-azetidinones[8,9]. Hence, the first position of indole was used as target for chemical modification by incorporating azetidinones. The structures of the synthesized compounds have been established by chemical tests, IR, ^{1}HNMR spectral data. Since titled compounds contain two biologically important heterocyclic rings with diverse medicinal value, synthesized compounds were screened for antimicrobial, antitubercular and antiinflammatory activities.

Melting points of the synthesized compounds were determined using open capillary tubes on an electrically heated block and are uncorrected. IR absorption spectra of synthesized compounds were determined on a FTIR-8300 (Shimadzu), KBr Press at Manipal College of Pharmaceutical Sciences, Manipal. ^{1}HNMR spectra were determined on a Gemini-200 MHz Spectrometer at the Indian Institute of Chemical Technology, Hyderabad. Necessary permission for conducting animal experiments was obtained from the Institutional Animal Ethics Committee (IAEC), KMC, Manipal, vide letter number: IAEC/KMC/05/2003-2004, dated 1st March 2004.

Ethyl-N-indolyl acetate (1), N-indolyl acetyl hydrazine (2) and N-(4-methyl benzylidine hydrazidomethyl)-indole (3e) were prepared as reported earlier[1]. Similarly other Schiff's bases (3a-n) were prepared and characterized. To a solution of 3e (0.01 mol) in dry n-hexane (30 ml), triethylamine (0.01 mol) was added. To this, chloroacetyl chloride (0.02 mol) was added drop by drop with stirring. The mixture was then refluxed for 3 h. The triethylamine hydrochloride which was formed as solid was removed by filtration. The filtrate and washings were mixed and concentrated under reduced pressure. The residue obtained was washed with petroleum ether (40-60°) to remove the unreacted Schiff's bases. The solid was then crystallized from aqueous ethanol. Solvent system used for TLC was n-hexane:methanol (95:5), Yield: 62%, mp: 188°, IR (KBr): 3413.4 cm^{-1}(-NH-), 1604 cm^{-1} (>C=O, CONH), 1705 cm^{-1} (>C=O,cyclic), ^{1}HNMR (DMSO): 3.35 (s, 2H, -N-CH_2), 6.8-7.9 (m,10H,Ar-H), 2.2 (m,3H, -C-CH_3), 6.2 (m,1H,-CH-Cl),8.3 (bs,1H, -CONH). Likewise other 4-(substituted aryl)-1-(N-indolyl acetamidyl)-3-chloro-2-azetidinones were prepared by treating 3a-n with chloroacetyl chloride (scheme 1) and their physical data were given in Table 1.

Antibacterial activity of all the titled compounds was determined by the disc diffusion method against the gram-positive organisms coagulase positive *Staphylococcus* and

TABLE 1: PHYSICAL DATA OF THE SYNTHESIZED COMPOUNDS

Compd.	R	Molecular Formula	% Yield	Mp (°)	Rf Value
4a	H	$C_{19}H_{16}N_3O_2Cl$	61	167	0.65
4b	o-Cl	$C_{19}H_{15}N_3O_2Cl_2$	59	176	0.62
4c	m-NO_2	$C_{19}H_{15}N_4O_4Cl$	64	166	0.66
4d	p-OCH_3	$C_{20}H_{18}N_3O_3Cl$	58	179	0.71
4e	p-CH_3	$C_{20}H_{18}N_3O_2Cl$	62	188	0.66
4f	o-NO_2	$C_{19}H_{15}N_4O_4Cl$	63	146	0.67
4g	p-$N(CH_3)_2$	$C_{21}H_{21}N_4O_2Cl$	57	191	0.69
4h	p-Br	$C_{19}H_{15}N_3O_2ClBr$	61	183	0.61
4i	o-OH	$C_{19}H_{16}N_3O_3Cl$	54	180	0.72
4j	m,p-$(Cl)_2$	$C_{19}H_{14}N_3O_2Cl_3$	58	174	0.65
4k	p-Cl	$C_{19}H_{15}N_3O_2Cl_2$	60	182	0.69
4l	p-OH	$C_{19}H_{16}N_3O_3Cl$	66	198	0.59
4m	di,m,p-$(OCH_3)_3$	$C_{21}H_{22}N_3O_5Cl$	56	194	0.74
4n	m-OH	$C_{19}H_{16}N_3O_3Cl$	61	161	0.69

Rf- retention factor, mp- melting point

TABLE 2: ANTIMICROBIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS

Compd	COPS	Enterococci	K. pneumoniae	P. aeruginosa	E. coli	V. cholerae	C. albicans	A. niger
4a	-	-	-	-	-	14	-	-
4b	13	-	-	-	-	-	-	-
4c	-	-	-	-	-	-	-	-
4d	13	-	-	-	14	-	-	-
4e	12	-	-	-	-	-	-	-
4f	14	-	-	9	11	-	-	-
4g	15	-	-	10	-	-	15	7
4h	14	-	-	-	-	-	-	-
4i	-	-	-	14	-	-	-	-
4j	-	-	-	13	-	11	-	-
4k	14	-	-	-	-	-	-	-
4l	13	-	-	-	-	12	-	-
4m	13	-	-	10	-	-	-	-
4n	16	-	12	-	13	-	-	-
Std	24	25	19	19	18	19	19	23

COPS-Coagulase positive *Staphylococcus, K.pneumoniae- Klebsiella pneumoniae , P. aeruginosa- Pseudomonas aeruginosa, E.coli- Escherichia coli ,V.cholerae-Vibrio cholerae, C. albicans- Candida albicans, A. niger- Aspergillus niger.* Zone of inhibition in mm.

TABLE-3: ANTITUBERCULAR ACTIVITY OF THE SYNTHESIZED COMPOUNDS

Compound	Dilution (μg/ml)		
	10	50	100
4a	-ve	-ve	-ve
4b	-ve	-ve	-ve
4c	-ve	-ve	-ve
4d	-ve	-ve	-ve
4e	-ve	-ve	-ve
4f	-ve	-ve	-ve
4g	-ve	-ve	-ve
4h	-ve	-ve	-ve
4i	-ve	-ve	-ve
4j	-ve	-ve	-ve
4k	-ve	-ve	-ve
4l	-ve	-ve	-ve
4m	-ve	-ve	-ve
4n	-ve	-ve	-ve
LJ Medium(control)	+ve	+ve	+ve
DMSO(control)	+ve	+ve	+ve
Rifampin	-ve	-ve	-ve

-ve indicates no growth and +ve means growth observed.

Enterococci and gram-negative organisms *Escherichiea coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and Vibrio cholerae* at 500 μg/ml concentrations. The bacteria were subcultured on Mueller Hinton Agar medium. The petridishes were incubated at 37° for 48 h. Standard antibacterial drugs were also screened under similar conditions for comparison. Penicillin (1000 units/ml) was used as a standard for Gram positive and gentamycin (1000 units/ml) was used as a standard for Gram- negative microorganisms. The results were given in Table 2.

The antifungal activity of 2-azetidinones was carried out against the fungi *Candida albicans* and *Aspergillus niger* at 500 μg/ml concentration. The fungi were subcultured in Sabourad's dextrose agar medium. The fungal susceptibility testing was done by disc diffusion method using fluconazole (1000 units/ml) as standard. The petridishes were incubated for 24 h at 22° to 25°. The results were given in Table 2. The *in vitro* antitubercular activity of all the synthesized compounds (4a-n) were carried out against the H_{37} Rv strain of *Mycobacterium tuberculosis* at 10, 50, 100 μg/ml. Lowenstein-Jensen Medium (LJ medium) was used for the screening. LJ tubes with solvent DMSO used as control and rifampin used as standard drug. The results were given in Table 3.

Carrageenan-induced rat hind paw oedema screening method was used for the antiinflammatory study. Oedema was produced using type IV λ carrageenan. Six Wistar rats

TABLE 4: ANTIINFLAMMATORY ACTIVITY OF THE SYNTHESIZED COMPOUNDS

Drug	Dose (mg/kg)	Mean edema volume±SE (0-3 h)	% reduction in edema volume
Control	2 ml	0.89±0.18	-
Ibuprofen	200	0.25±0.9*	73
4a	200	0.31±0.11	66
4b	200	0.35±0.05	62
4c	200	0.4±0.89	56
4d	200	0.40±0.05	60
4e	200	0.21±0.04*	76
4f	200	0.37±0.12	57
4g	200	0.30±0.06	67
4h	200	0.4±0.89	56
4i	200	0.45±0.99	51
4j	200	0.25±0.4*	72
4k	200	0.28±0.4	69
4l	200	0.26±0.06*	71
4m	200	0.37±0.12	57
4n	200	0.40±0.05	60

*The results are significant at $P<0.05$.

of either sex weighing about (100-250 g) in each group were used. The paw volume was measured in a plethysmograph by $KMnO_4$ solution displacement. Ibuprofen was used as a standard and 0.5 % gum acacia mucilage used as a control group and rest of the groups were used for the test drugs. The % inhibition of paw edema volume was calculated by the formula, % inhibition=$(1-V_t/V_c)x100$, where Vt is the mean volume of the test drug, Vc is the mean volume of the control and the results were given in Table 4.

All compounds were in conformity with the structures envisaged. The structures were proved on the basis of spectral data. All titled compounds were found to be active at 10 µg/ml concentration against H_{37} RV strain of *Mycobacterium tuberculosis* .All the compounds except 4c, 4i and 4j showed almost same inhibitory action against Coagulase positive *Staphylococcus* but less than that of standard penicillin. 4i and 4j were also moderately active against *P. aeruginosa* in comparison to standard. Only compound 4 g was moderately active against *C. albicans,* when compared with the standard. Results also indicate that 4e showed higher and 4j and 4l showed equal antiinflammatory activity in comparison to that of standard ibuprofen.

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Spectrophotometric Estimation of Total Alkaloids from *Rauwolfia* Root Powder and Formulation.

K. PUNDARIKAKSHUDU*, C. J. BHATT AND R. K. PATEL

Shree S.K. Patel College of Pharmaceutical Education and Research, Ganpat Vidyanagar, Kherva-382 711, Mehsana.

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Total alkaloids (calculated as reserpine) from various samples of *Rauwolfia serpentina* root powder and its marketed formulation were estimated by spectrophotometric method using ion-pair complexation of acid dye (methyl orange) with the alkaloids. The complex was selectively ex-

***For correspondence**

E-mail: p_kilambi@yahoo.com