Digest Journal of Nanomaterials and Biostructures Vol. 4, No.2, June 2009, p. 361 – 367

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF SOME 1-(NICOTINYLAMINO) -2 SUBSTITUTED AZETIDINE-4 -ONES AS POTENTIAL ANTIBACTERIAL AGENTS

M.C.SHARMA[a*], N.K. SAHU [a], D.V.KOHLI [a,] S.C. CHATURVEDI[b]
SMITA.SHARMA[c]
[a]*Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutical Sciences, Dr. H. S. Gour Vishwavidyalaya, Sagar (M.P) – 470 003, India*
[b] *College of Pharmacy, IPS Academy Indore (M.P)-452001 India*
[C] *Yadunath Mahavidalya Bhind (M.P)-477001*

Some new 1-(nicotinylamino)-2 substituted azetidin 4-ones have been synthesized by condensation of nicotinic acid hydrazide with various aromatic or heterocyclic aldehydes to yield the Schiff bases. Cyclocondensation of schiffs bases with chloroacetyl chloride in the presence of triethylamine results in azetidinone derivatives. The structures of the newly synthesized compounds were confirmed by analytical IR NMR and mass spectral data. All the synthesized compounds have exhibited significant activity against the bacteria and fungi tested.

(Received April 29, 2009; accepted May 4, 2009)

Keywords: Azetidin derivatives, Antibacterial activity, Antitubercular activity

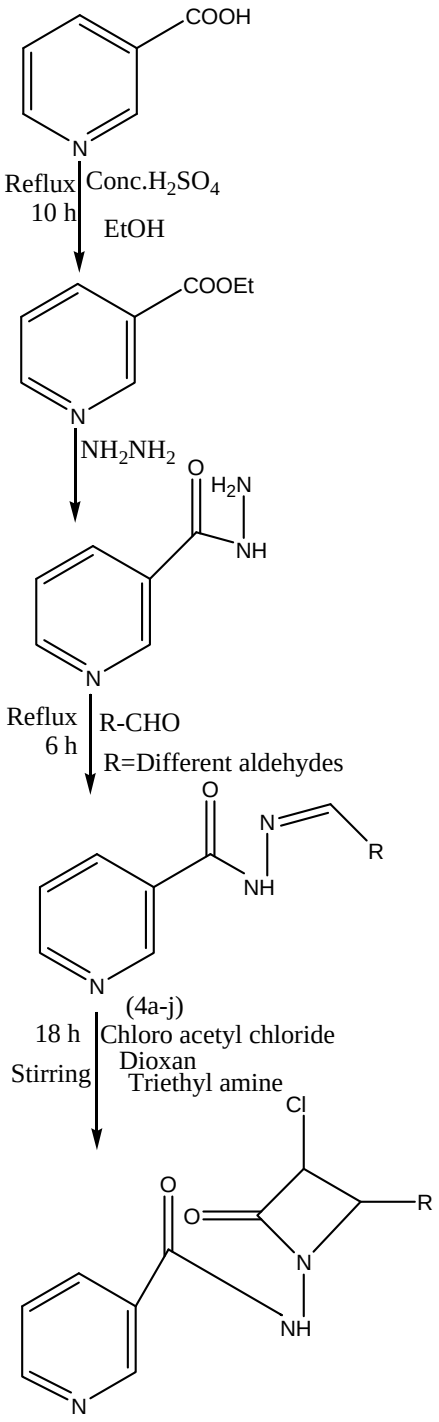
1. Introduction

The emergence and spread of antimicrobial resistance has become one of the most serious public health concerns across the world. Antimicrobial resistance refers to micro-organism that have developed the ability to inactivate, exclude or block the inhibitory or lethal mechanism of the antimicrobial agents **[1]** The acid-fast bacillus Mycobacterium tuberculosis is the causative agent of tuberculosis (TB). The tubercle bacillus is a slow growing organism, which does not elicit a sharp and massive reaction from the host. The tubercle bacillus does not produce any substance, which is toxic to the normal host. It acts as an irritating foreign body and tubercle formation can be produced by virulent, avirulent and nonpathogenic types. The tubercle bacillus is an intracellular parasite, and lives and grows within the host's tissue cells, macrophages and epithelial cells. The recent emergence of outbreaks of multidrug resistant tuberculosis (MDR-TB) poses a serious threat to the treatment of the disease **[**Antitubercular drugs available for the treatments were discovered in the period of 1945-1965. No new drugs were synthesized during the last few decades. There are millions of patients suffering from tuberculosis, this tells us about the necessity of searching for and synthesizing new highly active compounds with less side-effects. The search for new anti-tubercular drugs may be done using the Biochemical and Chemical methods **[2-3].** Azetidin-2-ones and their derivatives have been extensively investigated, considering the presence of β-lactam ring **[4]** moiety in their, structure just as in the case of highly popular β-lactam antibiotics. Nicotinic acid and its amides proved to be powerful antitubercular agents. Azetidin-2-ones have been reported to possess potent antitubercular, antibacterials**[5-6**], antifungal**[7-9**] activities, considering the significance of β-lactam ring system in azetidinones, a new series of azetidinone derivatives were synthesized characterized and their biological activities were investigated.

2. Experimental

All the chemicals used were of AR-grade purity. IR spectra were recorded on Perkin Elmer model 377 spectrophotometer in KBr pellets. ^{1}HNMR spectra were recorded on a Bruker DRX300 instrument. The FAB mass spectra were recorded on a JEOLSX102/DA-6000 Mass Spectrometer using argon/ xenon (6 kV, 10 mA) as the FAB gas. Analytical thin layer chromatography was performed using E. Merck silica gel G, 0.50 mm plates, (Merck No. 5700). The melting points were determined on an electric melting point apparatus in open capillaries and are uncorrected.

SCHEME



N-(3-chloro-2-aryl-4-oxoazetidin-1-yl)-nicotinamide
5a-j

2.2. Methods

2.2.1 Preparation of ethyl nicotinate (2) from nicotinic acid (1) :

Nicotinic acid (0.01 mol) was refluxed with sulphruric acid (50 ml) and absolute alcohol (115 ml) for 10 h. and the mixture was cooled to the room temperature and poured on to the crushed ice. The mixture was then made strongly alkaline by the addition of ammonia solution. The resulting mixture was extracted with ether solvent. The ether was then distilled off and the resultant liquid was recovered.

2.2.2 Preparation of nicotinic acid hydrazide (3) :

Ethyl nicotinate (0.01 mol) was condensed with hydrazine hydrate by maintaining the reaction temperature of 0^o. This resulted in solid nicotinic acid hydrazide. The resultant nicotinic acid hydrazide was recrystillised from warm ethanol.

2.2.3 Preparation of Schiff bases (4a-j):

Nicotinic acid hydrazide (0.01 mol) was refluxed were various aromatic / heterocyclic aldehydes (0.02 mol) in the presence of sulphuric acid for 6 h. The reaction mixture was then poured into the crushed ice. The resultant solid was washed with distilled water, dried in vacuum and recrystallised from warm ethanol.

2.2.4 Preparation of derivates of N-(3-Chloro-2 aryl-4-oxazetidin-1-yl) nicotinamide (5a-j) :

The Schiff bases (0.01 mol) were condensed with chloroacetylchloride (0.025 mol) and triethylamine (0.025 mol) in dry dioxane (50 ml). The mixture was stirred continuously for 20 h. The resulting contents were kept at room temperature for 18 h to complete the reaction. The contents were then poured on to the crushed ice. The solid material was filtered, washed with distilled water and recrystallised from warm ethanol.

N-[3-chloro-2-(2-nitrophenyul)-4-oxoazetidin-1-yl] nictotinamide:
Yield 72%, m.p. 234^o. IR (KBr) cm^{-1}. 1712.2 (c=0), 1564.5 (c=c), 2975.2 (C-H), 3380.1 (N-H). ^{1}HNMR δppm : 3.07 (s, 1H, CH-Cl), 5.38-5.40 (s, 1H, CH), 7.01-9.45 (m, 8H-Ar). MS m/z : 346 (M^+)148, 136, 120, 106.
N-(3-chloro-2-(4-dimethylamino) phenyl)-4-oxoazetidin-1-yl) nicotinamide:
Yield 69%, m.p. 241^o. IR (KBr) cm^{-1} : 1710.5 (C=0), 1600.1 (C=C), 3029.9 (C-H), 3403.6 (N-H). ^{1}HNMR δppm : 2.52 (s, 1H, CH-Cl), 5.0 – 5.01 (s, 1H, CH), 7.60-7.62 (s, 1H, NH), 6.95 – 9.65 (m, 8H-Ar) 3.45 – 3.48 (s, 6H, Ar-CH_3). MS m/z 344 (M+) 146, 136, 120, 106.
N-(3-chloro-2-(3, 4, 5-trimethoxylphenyl)-4-oxoazetidin-1-yl) nicotinamide:
Yield 60%, m.p. 237^o. IR (KBr) cm^{-1} 1695.5 (C=0), 1576.9 (C=C), 2939.5 (C-H), 3404.5 (N-H). ^{1}HNMR δppm : 3.07 (s, 1H, CH-Cl), 5.34-5.35 (s, 1H, CH), 3.46 (s, 1H, NH), 6.98-9.20 (m, 8H-Ar), 3.78 (s, 9H, 0CH_3) MS m/z : 391 (M^+) 193, 136, 120, 106.
N-(3-chloro-2-(phenyl)-4-oxoazetidin-1-yl) nicotinamide:
Yield 72 %, m.p. 201-203^o. IR (KBr) cm^{-1} 1682.5 (C=0), 1574 (C=C), 2979.5 (C-H), 3411.1 (N-H). ^{1}HNMR δppm : 3.11 (s, 1H, CH-Cl), 5.31 (s, 1H, CH), 3.41 (s, 1H, NH), 6.82-9.20 (m, 9H-Ar), MS m/z : 301.
N-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl) nicotinamide:
Yield 70 %, m.p. 262-264^o. IR (KBr) cm^{-1} 1702.5 (C=0), 1604 (C=C), 2965.5 (C-H), 3417 (N-H). ^{1}HNMR δppm : 3.14 (s, 1H, CH-Cl), 5.37 (s, 1H, CH), 3.48 (s, 1H, NH), 6.80-9.13 (m, 8H-Ar), MS m/z : 391 (M^+) 335.
N-(3-chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-yl) nicotinamide:

Yield 65 %, m.p. 226-229^{o}. IR (KBr) cm^{-1} 1710.5 (C=0), 1591 (C=C), 2960.5 (C-H), 3406 (N-H). ^{1}HNMR δppm : 3.07(s, 1H, CH-Cl), 5.30(s, 1H, CH), 3.42 (s, 1H, NH), 6.80-9.01 (m, 8H-Ar), MS m/z : 391 (M^{+}) 335.10

N-(3-chloro-2-(Furfuryl)-4-oxoazetidin-1-yl) nicotinamide:

Yield 59 %, m.p. 268-270^{o}. IR (KBr) cm^{-1} 1700.5 (C=0), 1598 (C=C), 2968.5 (C-H), 3424 (N-H). ^{1}HNMR δppm : 3.09(s, 1H, CH-Cl), 5.35(s, 1H, CH), 3.48 (s, 1H, NH), 6.80-9.01 (m, 9H-Ar), MS m/z : 391 (M^{+}) 305.

3. Antimicrobial activity

The antibacterial activity 9 of the test compounds was tested against *B. subtilis, E. Coli. S. aureus* and *P. aeruginosa* using tryptone Soya agar medium. The antifungal activity of the compounds was tested against *A. niger* and *C. albicans* using Sabour and dextrose agar medium. The sterilized**[10-13]** (autoclaved at 120^{o} for 30 min) medium (40-50^{o}) was innoculated (0.001 ml/ ml of medium) with the suspension of microorganism and poured into a petri dish to give a depth of 3-4 mm. The paper impregnated with the test compounds (200 µg/ml in dimethylformamide) was placed on solidified medium. The plate were incubated for 1 h at room temperature and at 37^{o} for 24 h and 48 h for antibacterial and antifungal activities respectively. *Ampicillin* (10 µg/disc) and *greiscofulvin* 10 µg/disc) was used as standard for antibacterial and antifungal activity respectively. The observed value of zone of inhibition are presented in Table 2.

Table 1. Physical constants of the synthesized compounds 4a-j and 5a-j

Compound	**R**	**M.F.**	**Yield (%)**	**M.P.(^{o})**
4a	2-hydroxy-4-methoxy	$C_{14}H_{13}N_3O_3$	72	217-219
4b	4-chlorophenyl	$C_{13}H_{13}ON_3Cl$	78	244-246
4c	2-nitrophenyl	$C_{13}H_{10}N_4O_3$	80	218-220
4d	4-dimethylamino phenyl	$C_{16}H_{16}N_4O$	75	210-212
4e	2-chlorophenyl	$C_{13}H_{10}N_3Ocl$	68	193-195
4f	Phenyl	$C_{13}H_{11}N_3O$	62	175-177
4g	Furfuryl	$C_{11}H_9N_3O_2$	70	196-198
4h	2-hydroxyphenyl	$C_{13}H_{11}N_3O_2$	76	217-219
4i	4-methoxyphenyl	$C_{14}H_{13}N_3O_2$	80	234-236
4j	3,4,5-trimethoxyphenyl	$C_{16}H_{17}N_4O_4$	85	201-202
5a	2-hydroxy-4-methoxy phenyl	$C_{16}H_{14}C1N_3O_4$	66	246-248
5b	4-chlorophenyl	$C_{15}H_{11}Cl_2N_3O_2$	70	262-264

5c	2-nitrophenyl	$C_{15}H_{11}C1N_4O_4$	72	234-236
5d	4-dimethylaminophenyl	$C_{15}H_{17}C1N_4O_2$	69	241-243
5e	2-chlorophenyl	$C_{15}H_{11}C1_2N_3O_2$	65	226-229
5f	Phenyl	$C_{15}H_{11}C1N_3O_2$	64	201-203
5g	Furfural	$C_{13}H_{12}C1N_3O_3$	59	268-270
5h	2-hydroxyphenyl	$C_{15}H_{12}C1N_3O_2$	65	230-232
5i	4-methoxyphenyl	$C_{16}H_{14}C1N_3O_3$	70	241-243
5j	3,4,5 – trimethoxyphenyl	$C_{18}H_{19}C1N_3O_5$	60	235-237

Table 2. Antimocrobial activity of the compounds 5a-j.

Compounds	Zone of Inhibition in (mm)					
	S. Aureus	B. Subtillis	E. Coli	P. aeroginosa	A. niger	C. albicana
5a	12	14	13	12	09	08
5b	13	15	14	13	14	13
5c	11	13	12	11	10	09
5d	12	14	13	12	11	10
5e	10	12	11	10	13	12
5f	13	15	14	13	10	09
5g	09	11	10	09	12	11
5h	08	10	09	08	11	10
5i	13	15	14	13	09	08
5j	12	14	13	12	10	09
Ampicillin	14	16	15	14	-	-
Greiscofulvin	-	-	-	-	15	14
DMF	-	-	-	-	-	-

Table 3. Antitubercular activity of the compounds 5a-j

Compounds	**Concentration in µg/ml**		
	100	10	1
5a	-	+	++
5b	-	+	++
5c	-	+	++
5d	-	-	++
5e	-	+	++
5f	-	+	++
5g	-	+	++
5h	-	+	++
5i	-	+	++
5j	-	-	++
Isoniazid	-	+	++
Control	++	++	++

(-) – Indicated no growth, (+) indicated growth less than 20 colonies, (++) indicated growth more than 20 colonies.

3.1 Antitubercular activity

The test compounds were tested for antitubercular activity (in 0.001ml/mol µg amount) against human strain H_{37} RV of *M. tuberculi* Isoniazid was used as standard drug. The results are presented in Table 3.

4. Results and discussion

All the compounds exhibited significant antibacterial and antifungal activities. Good antibacterial activity was observed in 5a, 5b, 5d, 5f, 5i against *B. Subtilis* compounds 5a, 5b, 5d, 5i showed good activity against *S. aureus* compounds 5a, 5b, 5d, 5j showed significant activity against *P. aeruginosa* and whereas compounds 5b, 5f, 5i showed noticeable activity against *E. coli*. Compound 5b, 5d, 5e, 5f showed marked activity against *A-niger* and *C. albicans*. Compounds 5d, 5j showed significant antitubercular activity (10 µg amount) in comparison to others.

5. Conclusions

Conclusively, a variety of azetidine derivatives have been successfully synthesized in appreciable yields and screened in vitro for their antimicrobial activities against both strains of Gram-positive and Gram-negative bacteria.

Acknowledgement

The authors are thankful to CDRI, Lucknow for providing analytical and spectral data.

References

[1] Tolaro, K.; Tolaro, A.; Foundation of Microbiology, W.C. Brown Publisher, Dubuque, edition, 3, 1993, 326.
[2] Bloom, B. R.; Murray, C. J. L. Science 1992, 257, 1055.
[3] WHO report on the Tuberculosis Epidemic stop TB at the source. Tuberculosis Programme World Health Organization, Geneva, Switzerland, 1995.
[4] Kamyia, T., Hashimotoo, M., Nakaguchi, O., and Oka, T., Tetrahedron 35 (1979) Gilamann, H., Specter, M. J. Am. Chem. Soc., 65, 1943.
[5] Trivedi, Bhavin, Shah, V.H., Indian J. Chem. Soc., 1993, 70, 601.
[6] Udapi, R.H., Jeeson, M. and Bhatt, A.R., Indian J. Het. Chem., 1996, 6, 99.
[7] Vashi, B.S. and Shah, V.H., Indian J. Chem. Soc., 1996, 73, 491.
[8] Desai, N.C., Dipika, Shah, M.D. and Vyas, G.D. Indian J. chem., 2000, 39B.
[9] Mehta, D.S. and Shah, V.H., Indian Het. Chem., 2001, 11, 139.
[10] Trivedi, P.B., Undavia N.K., Dave, A.M., Indian J. chem., 1993, 32B, 760.
[11] Desai, Sunil B., Desai, P.B. and Desai K.R., Asia. J. Chem**.,** 1990, 1/4, 1431.
[12] Gillespie, S.H. in Medical Microbiology – Illustrated; Butterworth, Heinemann, London, 1994, 234.
[13] Hawkey, P.M. and Lewis, D.A., in; Medical Bacteriology – A Practical Approach Oxford University, Press, 1994, 181.