American Journal of Advanced Drug Delivery

www.ajadd.co.uk

Original Article

Synthesis and Antimicrobial Screening of Some Azetidine Derivatives

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Date of Receipt- 30/01/2014
Date of Revision- 11/02/2014
Date of Acceptance- 14/02/2014

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ABSTRACT

New series of N-(3-chloro-2-oxo-4-substituted-azetidine-1-yl) isonicotinamide derivatives were synthesized by the reaction of Schiff base with 2-chloroacetic acid. Synthesized compounds were evaluated for their Anti-bacterial activity against *staphylococcus aureus* and *Echerichia coli*, Antifungal activity against *C Albicans* and Anti-tubercular activity against *mycobacterium tuberculosis*. Synthesized compounds show significant activity against bacterial, fungal and mycobacterium strains. Their structures were established on the basis of elemental analysis, IR, 1H NMR and Mass Spectral data.

Keywords: INZ, Isoniazid, Azetidine, Antimycobacterial, TB.

INTRODUCTION

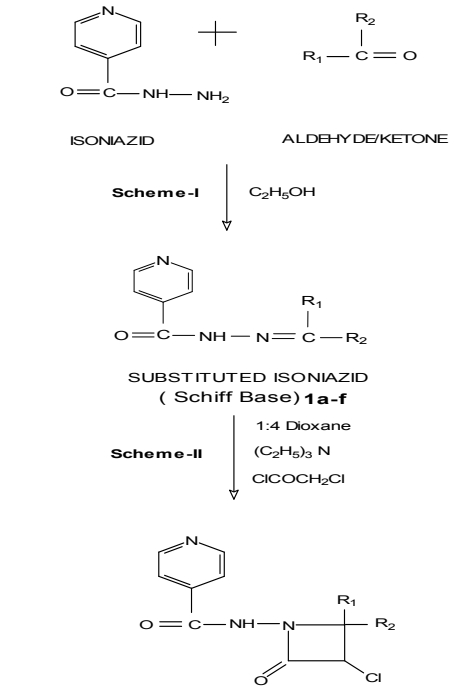
Natural, synthetic and semi synthetic antimicrobial agents have been used since a long time against the life threatening infectious diseases[1]. Deaths from bacterial and fungal infection have dropped currently, but still those are the major cause of death in the world.[2] Over the few past decades the bacterial resistance to antibiotics, anti-fungal and anti-tuberculotic drugs has become one of the most challenging problems in the infections treatments. Tuberculosis (TB) is a chronic grannulomatous disease[3] and world's oldest known infectious disease that kills three million deaths each year. The causative organism of disease is *mycobacterium tuberculosis*[4]. The urgency to develop new and effective drugs is due to the resistance development by strains against current medications and grooving problem of co-infection in immuno-compromised patients.[5,6]

Several research has been done and currently in progress to develop new and better chemical entity against infections. In addition, knowledge of specific constituents of the mycobacterial cell and their biochemical roll has advance considerably in current years and may permit a more rational approach to the design of new drugs acting on specific target[7]. Literature survey reveals that 2-oxo-azetidines have shown various biological activities along with antimicrobial activity.[8-16] In view of these findings some 2-oxo-azetidine derivatives of Isoniazid have been synthesized and evaluated for anti-bacterial, antifungal and anti-tubercular activity.

MATERIALS AND METHODS

All the chemicals used were purchased from E Merk, S D Fine and Loba Chem and were purified by established methods (whenever needed). Various Substituted Isoniazid (Schiff Base) derivatives were prepared according to the procedure outline in scheme-I. 2-oxo azetidine derivatives were synthesized by formation of imines (from Schiff base) and ketenes (from 2-chloro acetyl chloride) followed by cycloaddition of ketenes to imines, in the presence of 1:4 Dioxane, in a single step reaction. Melting points were determined by open capillary tube method and are uncorrected. Purity of synthesized compounds was checked by TLC plates (Silica Gel G) and visualized by iodine vapor. The infra red absorption spectra of the synthesized compounds were recorded using KBr disc on FTIR 8010 Shimadzu model. ^{1}H NMR spectra were recorded on Brucker Spectrospin DPX 300 spectrophotometer. Mass spectra were recorded on Jeol SR-102 FAB Mass spectrometer. CHN analyses of synthesized compounds were done on Perkin-Elmer-240 analyzer.

Scheme



N-(4,4-disubstituted-3-chloro-2-oxo-azetidine-1-yl) isonicotinamide

2a-f

General procedure for synthesis of substituted Isoniazid (Schiff Base), (1a-f)

A mixture of Isoniazid (0.01 mol), aldehyde/ketone (0.01 mol) and ethanol (30-35ml) were reflux for 3 Hrs. The reaction mixture were cooled at room temperature and allowed to stand for 5 hrs. Solid products were separated out, filtered, washed with ice-cold water, dried and recrystallized from ethanol.

General procedure for synthesis of 2-oxo-azetidine derivatives of Isoniazid, (2a-f)

Substituted Isoniazid, Schiff Base (0.01 mol) (1a-f) were dissolved in 1:4 Dioxane (20ml) with constant stirring, triethylamine (0.01 mol) was added followed by drop wise addition of 2-chloro acetyl chloride (0.01 mol). Content was stirred vigorously for 15 minutes and refluxed for 5 hrs. Mixture was cooled at room temperature, filtered, washed with ice cooled water, dried and recrystallized from ethanol. Physical Characteristics were shown in Table No.1.

2a: N-(4-methy-4-phenyl- 3-chloro-2-oxo-azetidine-1-yl) isonicotinamide

IR (KBr, cm^{-1}): 3310 (N-H Sec. Amide), 3050 (C-H Ar), 2870 (C-H alkyl) 1715 (C=O Cyclic β-Lactam), 1665 (C=N, Pyridine), 1620 (C=O acyclic). 1H NMR (DMSO-d_6 δ ppm) 8.6 (s, 1H, NH Amide), 7.8-8.4 (m, 4H, CH Pyridine), 7.1-4.2 (m, 5H phenyl), 4.1-4.2 (s, 1H, CH-Cl of β-Lactam), 2.6 (t, 3H, Methyl). Elemental analysis % found (% calculated): C-68.62 (68.57), H-4.89 (5.00), N-15.08 (15.00).

2b: N-(4, 4-diphenyl- 3-chloro-2-oxo-azetidine-1-yl) isonicotinamide

IR (KBr, cm^{-1}): 3325 (N-H Sec. Amide), 3050 (C-H Ar), 1715 (C=O Cyclic β-Lactam), 1665 (C=N, Pyridine), 1620 (C=O acyclic). 1H NMR (DMSO-d_6 δ ppm) 8.6 (s, 1H, NH Amide), 7.8-8.4 (m, 4H, CH Pyridine), 6.9-7.6 (m, 10H phenyl), 4.1-4.2 (s, 1H, CH-Cl of β-Lactam). Elemental analysis % found (% calculated): C-73.76 (73.68), H-4.49 (4.68), N-12.22 (12.28).

2c: N-(4- furfural- 3-chloro-2-oxo-azetidine-1-yl) isonicotinamide

IR (KBr, cm^{-1}): 3265 (N-H Sec. Amide), 2970 (C-H Ar), 1780 (C=O Cyclic β-Lactam), 1660 (C=N, Pyridine), 1610 (C=O acyclic) 1280 (C-O cyclic). 1H NMR (DMSO-d_6 δ ppm) 8.6 (s, 1H, NH Amide), 7.8-8.4 (m, 4H, CH Pyridine), 7.3-7.4 (m, 4H Ar), 5.8 (3H, furfural) 4.1-4.2 (s, 1H, CH-Cl of β-Lactam), 2.5 (s, 1H CH of β-Lactam). Elemental analysis % found (% calculated): C-61.05 (60.94), H-3.86 (3.91), N-16.52 (16.41).

2d: N-(4-pyridine- 3-chloro-2-oxo-azetidine-1-yl) isonicotinamide

IR (KBr, cm^{-1}): 3270 (N-H Sec. Amide), 2960 (C-H Ar), 1780 (C=O Cyclic β-Lactam), 1660 (C=N, Pyridine), 1610 (C=O acyclic) 1365 (CH-N). 1H NMR (DMSO-d_6 δ ppm) 8.6 (s, 1H, NH Amide), 7.7-8.4 (m, 8H, CH, Pyridine), 4.1-4.2 (s, 1H CH-Cl of β-Lactam), 2.5 (s, 1H CH of β-Lactam). Elemental analysis % found (% calculated): C-63.02 (62.92), H-4.24 (4.12), N-20.88 (20.97).

2e: N-(4-indole- 3-chloro-2-oxo-azetidine-1-yl) isonicotinamide

IR (KBr, cm^{-1}): 3310 (N-H Sec. Amide), 3020 (C-H Ar), 1780 (C=O Cyclic β-Lactam), 1660 (C=N, Pyridine), 1610 (C=O acyclic) 1290 (C-N Ar-amine) 780 (N-H wag sec amine). 1H NMR (DMSO-d_6 δ ppm) 8.6 (s, 1H, NH Amide), 7.8-8.4 (m, 4H, CH, Pyridine), 7.6 (m, 4H, CH phenyl) 7.2 (s, 1H indole), 6.3-6.5 (s, 1H, CH pyrrole) 4.1-4.3 (s, 1H CH-Cl of β-Lactam), 2.5 (s, 1H CH of β-Lactam). Elemental analysis % found (% calculated): C-66.76 (66.89), H-4.17 (4.26), N-18.25 (18.36).

2f: N-(4-thiophene- 3-chloro-2-oxo-azetidine-1-yl) isonicotinamide

IR (KBr, cm^{-1}): 3290 (N-H Sec. Amide), 2970 (C-H Ar), 1780 (C=O Cyclic β-Lactam), 1660 (C=N, Pyridine), 1610 (C=O acyclic) 1330 (C-S). 1H NMR (DMSO-d_6 δ ppm) 8.6 (s, 1H, NH Amide), 7.8-8.4 (m, 4H, CH, Pyridine), 7.2-7.3 (t, 3H, thiophene) 4.1-4.3 (s, 1H CH-Cl of β-Lactam), 2.5 (s, 1H CH of β-Lactam). Elemental analysis % found (% calculated): C-57.28 (57.35), H-3.52 (3.68), N-15.32 (15.44).

Antimicrobial Activity

All the synthesized compounds were evaluated for their *in vitro* antimicrobial activity against gram positive bacteria *staphylococcus aureus* (ATCC-24392), the gram negative bacteria *Echerichia coli* (ATCC-24391) in nutrient agar media,[12] Fungi *C Albicans* (ATCC-436) in sabouraud dextrose medium[13] and *mycobacterium tuberculosis* (ATTC-27286) in tween-albumin medium[14]. The zone of inhibition values were determined and compared with well known (standard) antibacterial (Ofloxacin), antifungal (Ketoconazole) and antituber-culotic (Isoniazid) drugs. Table: 2 shows data obtained from the biological screening of synthesized compounds and reference drugs.

RESULTS AND DISCUSSION

Chemistry

Yield of synthesized compounds were found to be satisfactory. The purity of synthesized compounds and completion of reactions were checked by TLC on silica Gel G plates in the solvent system methyl chloride: methanol (8:2 v/v) and visualized spots in iodine vapor. Proposed structures were confirmed by Spectral and microanalysis data. 1H NMR (δ ppm) spectra shows signals at 4.1-4.3 (CH-Cl of β-Lactam), 8.6 (N-H Secondary Amide) and 7.8-8.4 (C-H Pyridine). Presences of various functional groups and heteroatom were supported by the IR and Mass spectral data. Further elemental analysis data were also found in agreement with calculated values from proposed structures.

Antimicrobial Activity

Antimicrobial screening data of synthesized compounds showed good to moderate activity, against bacterial, fungal and mycobacterium strain, as compared to reference drug. Compound 2a showed moderate activity against all strains. Compound 2b showed good activity against *E Coli* while showed moderate activity against *S. aureus, C. Albicans* and *mycobacterium tuberculosis*. Compound 2c and 2f showed good activity against all strains. Compound 2d and 2e showed excellent antibacterial, antifungal and anti tuberculotic activity against tested strains. The antimicrobial activity is due the presence of pharmacological active β-Lactam ring and increased by the addition phenyl moiety/heterocyclic compounds at 4 position of β-Lactam ring. Amongst these 2e showed highest activity against *mycobacterium tuberculosis* as compare to other synthesized compounds, is due to the presence the indole moiety at 4 position of azetidine ring.

CONCLUSION

From the above result and discussion it is concluded that the synthesized compounds posses' good anti-bacterial, anti-fungal and anti-mycobacterium activity. Process optimization, clinical safety and dosage form development of these compounds are needed. Furthermore development of new azetidine derivative by this scheme is highly desirable.

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Table 1. Physical characteristics of synthesized compounds

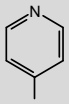
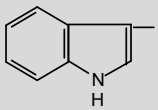
Compound	R_1	R_2	Molecular formula	Yield (%)	m. p. (°C)	Molecular weight	Rf
2a	C_6H_5	CH_3	$C_{16}H_{14}N_3O_2$	56	278	280	0.5
2b	C_6H_5	C_6H_5	$C_{21}H_{16}N_3O_2$	59	265	342	0.4
2c		H	$C_{13}H_{10}N_3O_3$	68	235	256	0.7
2d		H	$C_{14}H_{11}N_4O_2$	62	260	267	0.7
2e		H	$C_{17}H_{13}N_4O_2$	64	295	305	0.8
2f		H	$C_{13}H_{10}N_3O_2S$	59	278	272	0.6

Table 2. Antimicrobial screening data of synthesized compounds

Compounds	Zone of Inhibition (in mm) at concentration of 20 µg/mL)			
	S. aureus	*E. coli*	*C. albicans*	*M. tuberculosis*
2a	11	17	23	27
2b	13	19	27	29
2c	14	17	27	31
2d	19	21	29	33
2e	16	23	32	36
2f	18	19	27	28
Ofloxacin	12	16	-----	-----
Ketoconazole	-----	-----	26	-----