Research Article

Synthesis, Antimicrobial and Antitubercular Activities of Some Novel Trihydroxy Benzamido Azetidin-2-one Derivatives

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Abstract

Purpose: To synthesize and characterize novel trihydroxy benzamido azetidin-2-one derivatives and screen them for antimicrobial and antitubercular activities.

Methods: A series of novel 4-aryl-3-chloro-N-(3,4,5-trihydroxy benzamido)-2-azetidinones, 3a-o, were synthesized by reacting various Schiff bases of galloyl hydrazide, 2a-o, with chloroacetyl chloride in the presence of dioxan and triethylamine. Schiff bases of galloyl hydrazide, 2a-o, were synthesized from galloyl hydrazide. The newly synthesized compounds were characterized by infrared spectroscopy (IR), mass spectroscopy (MS) and proton nuclear magnetic spectroscopy (\( ^1 \text{H} \) NMR) and elemental analysis; they were also screened for in vitro antibacterial, antifungal and antitubercular activities. Ciprofloxacin and ketoconazole were used as reference standards for antibacterial and antifungal activities, respectively, while isoniazid was used as reference standard for antitubercular activity.

Results: Compounds 3f, 3g and 3o with chlorophenyl group and compound 3k with 4-dimethyl amino phenyl group exhibited good antimicrobial activity. Also, compounds 3f, 3g, 3k and 3o showed antitubercular activity with minimum inhibitory concentration (MIC) values equivalent to the standard drug (isoniazid). MIC values of 3f, 3g, 3k and 3o were 0.76, 0.57, 0.62 and 0.83 \( \mu \text{g/ml} \), respectively, while the MIC of isoniazid was 0.56.

Conclusion: We report the successful synthesis, spectral characterization, as well as in vitro antimicrobial and antitubercular evaluation of a series of novel trihydroxy benzamido azetidin-2-one derivatives. The work shows the emergence of new antimicrobial and antitubercular compounds.

Keywords: Azetidinone, Schiff bases, Synthesis, Antimicrobial activity, Antitubercular activity.
INTRODUCTION

Tuberculosis, one of the most common infections, is caused by *Mycobacterium tuberculosis*. According to the World Health Organization (WHO), nearly one third of the world's population has been exposed to the tuberculosis pathogen [1]. Annually, 8 million people become ill with tuberculosis and 2 million people die from the disease worldwide. Every 15 seconds, someone dies of tuberculosis [2]. There are a number of known factors that make people more susceptible to tuberculosis infection worldwide, the most important of which is human immunodeficiency virus (HIV). The association of tuberculosis with HIV infection is so dramatic that in some cases, nearly two-third of the patients diagnosed with the tuberculosis are also HIV-1 seropositive [3]. Smoking more than 20 cigarettes a day also increases the risk of tuberculosis by two- to four times [4].

Following the discovery of isoniazid, there have been no new classes of antitubercular drugs in past 40 years [5]. Moreover, there has been a recent and disturbing increase in the number of tubercular cases that are caused by organisms which are resistant to the first-line drugs such as isoniazid, rifampicin, ethambutol, streptomycin and pyrazinamide [6]. The Global Alliance for Tuberculosis (GATB) drug development was established to address this need [7]. Its top priority is the development of a new agent that will shorten the duration of chemotherapy from the current 6 – 8 months to two months or less. Also new drugs with activity against mycobacterium drug-resistant tuberculosis and latent tuberculosis are needed.

The addition of a beta-lactamase inhibitor to amoxicillin greatly improves its *in vitro* activity against *M. tuberculosis* [8]. Gallic acid is a naturally existing antioxidant [9] which occurs along with tannins in most plant species. It possesses various biological activities such as analgesic [10], antimicrobial [11], antineoplastic [12] and antityrosinase [13].

Azetidinone is chemically 2-Azacyclobutanone which is an integral part of penicillin. Ezetimibe, an azetidinone, represents the first new approach to lipid lowering therapy in more than a decade [14]. Moreover, this heterocycle is associated with muscle relaxant [15], antitubercular [16] and antiinflammatory [17] activities. Considering the above pharmacological activities of both gallic acid and azetidinones we thought that it would be worthwhile to incorporate the azetidinone ring into gallic acid to have synergistic effect.

EXPERIMENTAL

Equipment

Reactions were monitored by thin layer chromatography (TLC) on precoated silica gel plates obtained from E. Merck and Co. Melting points were determined using Veego digital melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer FTIR Spectrometer. $^1$H NMR spectra were measured with a Bruker Spectrometer [400 MHz] in CDCl$_3$ using tetramethyilsilane (TMS) TMS as an internal standard. Mass spectra were obtained with LC-MSD Trap- SL 2010A Shimadzu. Elemental analysis was determined on a Perkin Elmer - 240 CHN elemental analyzer.

Materials

All the chemicals used were of analytical reagent grade and obtained from Ranbaxy Fine Chemicals Ltd, India. Muller-Hinton and Sabouraud dextrose agars were obtained from Hi-Media Ltd, India. The bacterial strains were provided by School of Biotechnology, SRM University, Kattankulathur, India. Ciprofloxacin and ketoconazole were obtained from M/S Orchid Chemicals and Pharmaceuticals Limited, Chennai, India.
Methods

All the compounds were synthesized according to the scheme shown in Figure 1. The purity of the compounds was determined on TLC plates using silica gel G as a stationary phase and iodine vapors as visualizing agent.

Synthesis of 3,4,5-trihydroxy benzoic acid hydrazide from propyl gallate (1a)

Propyl gallate (0.01 mol) in ethanol and hydrazine hydrate (0.01 mol) were refluxed for 6 h. The excess of solvent was distilled off under reduced pressure using a vacuum pump. The cold residual mass was washed with distilled water, filtered and dried. The
crude product obtained was recrystallised from methanol to yield galloyl hydrazide 1a. Yield: 85%, Colour: Pale white, \( R_f \) value: 0.61, Mp: 167 °C.

**General procedure for synthesis of N-substituted arylidene galloyl hydrazide (2a-o)**

Equimolar quantities of galloyl hydrazide 1a and various aromatic aldehydes in ethanol were refluxed for 6 h. The completion of reaction was monitored on silica gel G precoated TLC plates using hexane and ethyl acetate (1:1) as an eluent and observed under UV light. The resultant mixture was poured into ice cold water. The crude Schiff bases were washed, filtered, dried and recrystallised from ethanol.

**N'-benzylidene galloyl hydrazide (2a):** Yield: 73%, Colour: Pale white, \( R_f \) value: 0.61, Mp: 170-172 °C. IR (KBr in cm\(^{-1}\)): 3407 (NH Str); 3313 (Ar-OH Str); 2987 (Ar-CH Str); 1693 (C=O Str); 1480 (C=N Str); 1446 (CO-NH Str). \(^1\)H NMR (DMSO, \( \delta \) ppm): 8.35 (s, 1H, CH=N), 7.28-7.95 (m, 7H, Ar-H), 4.34 (s, 1H, NH), 2.83 (s, 3H, 3(OH)).

**N'- (2-hydroxy) benzylidene galloyl hydrazide (2b):** Yield: 65%, Colour: Pale yellow, \( R_f \) value: 0.61, Mp: 168-169 °C. IR (KBr in cm\(^{-1}\)): 3411 (NH Str); 3332 (Ar-OH Str); 2971 (Ar-CH Str); 1675 (C=O Str); 1476 (C=N Str); 1438 (CO-NH Str). \(^1\)H NMR (DMSO, \( \delta \) ppm): 8.37 (s, 1H, CH=N), 6.96-7.95 (m, 6H, Ar-H), 4.31 (s, 1H, NH), 2.76 (s, 4H, 4(OH)).

**N'- (2-hydroxy-3-methoxy) benzylidene galloyl hydrazide (2c):** Yield: 77%, Colour: Pale yellow, \( R_f \) value: 0.61, Mp: 171-172 °C. IR (KBr in cm\(^{-1}\)): 3331 (NH Str); 3310 (Ar-OH Str); 2987 (Ar-CH Str); 1678 (C=O Str); 1480 (C=N Str); 1444 (CO-NH Str). \(^1\)H NMR (DMSO, \( \delta \) ppm): 8.25-9.12 (m, 5H, Ar-H), 8.41 (s, 1H, CH=N), 4.98 (s, 1H, NH), 3.9 (s, 3H, Ar-OCH\(_3\)), 3.48 (s, 4H, 4(OH)).

**N'- (3-hydroxy) benzylidene galloyl hydrazide (2d):** Yield: 79%, Colour: Pale brown, \( R_f \) value: 0.61, Mp: 170-171 °C. IR (KBr in cm\(^{-1}\)): 3334 (NH Str); 3312 (Ar-OH Str); 2981 (Ar-CH Str); 1672 (C=O Str); 1470 (C=N Str); 1442 (CO-NH Str). \(^1\)H NMR (DMSO, \( \delta \) ppm): 8.91-9.52 (m, 6H, Ar-H), 8.37 (s, 1H, CH=N), 4.41 (s, 1H, NH), 3.67 (s, 4H, 4(OH)).

**N'- (4-hydroxy) benzylidene galloyl hydrazide (2e):** Yield: 82%, Colour: Pale white, \( R_f \) value: 0.61, Mp: 172-174 °C. IR (KBr in cm\(^{-1}\)): 3318 (NH Str); 3309 (Ar-OH Str); 2973 (Ar-CH Str); 1674 (C=O Str); 1476 (C=N Str); 1438 (CO-NH Str). \(^1\)H NMR (DMSO, \( \delta \) ppm): 8.48-9.07 (m, 6H, Ar-H), 8.34 (s, 1H, CH=N), 4.4 (s, 1H, NH), 2.38 (s, 4H, 4(OH)).

**N'- (2-chloro) benzylidene galloyl hydrazide (2f):** Yield: 79%, Colour: Pale white, \( R_f \) value: 0.61, Mp: 168-170 °C. IR (KBr in cm\(^{-1}\)): 3410 (NH Str); 3304 (Ar-OH Str); 2994 (Ar-CH Str); 1758 (C=O Str); 1438 (C=N Str); 1419 (CO-NH Str); 762 (C-Cl Str). \(^1\)H NMR (DMSO, \( \delta \) ppm): 8.54-9.67 (m, 5H, Ar-H), 8.36 (s, 1H, CH=N), 7.73 (s, 1H, CH-Cl), 4.28 (s, 1H, NH), 2.79 (s, 3H, 3(OH)).

**N'- (3-chloro) benzylidene galloyl hydrazide (2g):** Yield: 81%, Colour: Pale yellow, \( R_f \) value: 0.61, Mp: 171-173 °C. IR (KBr in cm\(^{-1}\)): 3335 (NH Str); 3318 (Ar-OH Str); 2983 (Ar-CH Str); 1758 (C=O Str); 1437 (C=N Str); 1422 (CO-NH Str); 760 (C-Cl Str). \(^1\)H NMR (DMSO, \( \delta \) ppm): 8.32 (s, 1H, CH=N), 7.48-8.52 (m, 5H, Ar-H), 7.64 (s, 1H, CH-Cl), 4.38 (s, 1H, NH), 2.92 (s, 3H, 4(OH)).

**N'- (2-nitro) benzylidene galloyl hydrazide (2h):** Yield: 80%, Colour: Pale brown, \( R_f \) value: 0.61, Mp: 170-171 °C. IR (KBr in cm\(^{-1}\)): 3328 (NH Str); 3302 (Ar-OH Str); 2974 (Ar-CH Str); 1749 (C=O Str); 1534 (C-NO\(_2\) Str); 1441 (C=N Str); 1421 (CO-NH Str). \(^1\)H NMR (DMSO, \( \delta \) ppm): 7.23-8.51 (m, 6H, Ar-H), 8.31 (s, 1H, CH=N), 4.49 (s, 1H, NH), 2.63 (s, 3H, 3(OH)).
N’- (3-nitro) benzylidene galloyl hydrazide (2i): Yield: 72 %, Colour: Pale yellow, Rf value: 0.61, M.p.; 171-172°C. IR (KBr in cm⁻¹): 3400 (NH Str); 3312 (Ar-OH Str); 1685 (C=O Str); 1535 (C-NO₂ Str); 1480 (C=N Str); 1437 (CO-NH Str). ¹H NMR (DMSO, δ ppm): 7.41-8.69 (m, 6H, Ar-H), 8.41 (s, 1H, CH=N), 4.52 (s, 1H, NH), 2.56 (s, 3H, 3(OH)).

N’- (4-nitro) benzylidene galloyl hydrazide (2j): Yield: 85 %, Colour: Pale brown, Rf value: 0.61, M.p.; 171-172°C. IR (KBr in cm⁻¹): 3405 (NH Str); 3315 (Ar-OH Str); 2987 (Ar-CH Str); 1685 (C=O Str); 1538 (C-NO₂ Str); 1480 (C=N Str); 1432 (CO-NH Str). ¹H NMR (DMSO, δ ppm): 8.31-9.33 (m, 6H, Ar-H), 8.34 (s, 1H, CH=N), 4.52 (s, 1H, NH), 2.67 (s, 3H, 3(OH)).

N’- (4-dimethylamino) benzylidene galloyl hydrazide (2k): Yield: 77 %, Colour: Pale white, Rf value: 0.61, M.p.; 171-172°C. IR (KBr in cm⁻¹): 3395 (NH Str); 3331 (Ar-OH Str); 2997 (Ar-CH Str); 1668 (C=O Str); 1535 (C-NO₂ Str); 1410 (C=N Str); 1441 (CO-NH Str). ¹H NMR (DMSO, δ ppm): 8.18-9.33 (m, 4H, Ar-H), 8.28 (s, 1H, CH=N), 4.89 (s, 1H, NH), 2.21 (s, 3H, 3(OH)).

N’- (3,4,5-trimethoxy) benzylidene galloyl hydrazide (2l): Yield: 81 %, Colour: Pale yellow, Rf value: 0.61, M.p.; 168-169°C. IR (KBr in cm⁻¹): 3395 (NH Str); 3333 (Ar-OH Str); 2972 (Ar-CH Str); 1670 (C=O Str); 1614 (C=O-C Str); 1475 (C=N Str); 1433 (CO-NH Str). ¹H NMR (DMSO, δ ppm): 7.85-9.77 (m, 6H, Ar-H), 8.36 (s, 1H, CH=N), 4.18 (s, 1H, NH), 3.82 (s, 3H, 3(OCH₃)).

N’- (3,4-dimethoxy) benzylidene galloyl hydrazide (2m): Yield: 82 %, Colour: Pale brown, Rf value: 0.61, M.p.; 168-169°C. IR (KBr in cm⁻¹): 3405 (NH Str); 3333 (Ar-OH Str); 2987 (Ar-CH Str); 1670 (C=O Str); 1614 (C=O-C Str); 1475 (C=N Str); 1439 (CO-NH Str). ¹H NMR (DMSO, δ ppm): 8.16-9.12 (m, 5H, Ar-H), 8.37 (s, 1H, CH=N), 4.71 (s, 1H, NH), 3.82 (s, 6H, (OCH₃)₂), 2.41 (s, 3H, 3(OH)).

N’- (4-methoxy) benzylidene galloyl hydrazide (2n): Yield: 87 %, Colour: Pale yellow, Rf value: 0.61, M.p.; 171-173°C. IR (KBr in cm⁻¹): 3405 (NH Str); 3333 (Ar-OH Str); 2972 (Ar-CH Str); 1670 (C=O Str); 1614 (C=O-C Str); 1475 (C=N Str); 1433 (CO-NH Str). ¹H NMR (DMSO, δ ppm): 8.54-9.77 (m, 6H, Ar-H), 8.36 (s, 1H, CH=N), 4.18 (s, 1H, NH), 3.82 (s, 3H, OCH₃), 2.74 (s, 3H, 3(OH)).

N’- (4-chloro) benzylidene galloyl hydrazide (2o): Yield: 83 %, Colour: Pale white, Rf value: 0.61, M.p.; 179 - 181°C. IR (KBr in cm⁻¹): 3405 (NH Str); 3328 (Ar-OH Str); 2976 (Ar-CH Str); 1666 (C=O Str); 1624 (C=O-C Str); 1435 (C=N Str); 1424 (CO-NH Str); 763 (C-Cl Str). ¹H NMR (DMSO, δ ppm): 8.39-9.55 (m, 5H, Ar-H), 8.36 (s, 1H, CH=N), 7.7 (s, 1H, CH-Cl), 4.15 (s, 1H, NH), 2.14 (s, 3H, 3(OH)).

General procedure for Synthesis 4-Aryl-3-chloro-N-(3,4,5-trihydroxy benzamido)-2-azetidinones (3a-o)

A mixture of Schiff base (0.002 mol) and triethyl amine (0.004 mol) were dissolved in 1,4-Dioxan (50 ml). To this, well stirred cold solution of chloroacetyl chloride (0.004 mol) was added drop wise for 20 min, then stirred for further 3 h and left at room temperature for 48 h [18]. The resultant mixture was concentrated, cooled and then poured into ice cold water filtered off, dried and recrystallised from methanol.
(3R, 4S)-3-Chloro-4-(2-hydroxy phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3b): Yield: 73 %, Colour: Pale yellow, R<sub>f</sub> value: 0.78, M.p.; 198 °C. Anal. calcd. for C<sub>16</sub>H<sub>13</sub>CIN<sub>2</sub>O<sub>5</sub> (364): C, 54.34; H, 7.68; N, 6.67 %. Found: C, 54.38; H, 7.65; N, 6.69 %. IR (KBr in cm<sup>-1</sup>): 3613 (Ar-Oh Str); 2977 (Ar-CH Str); 1755 (C=O Str); 1591 (CONH Str); 1488 (CH-N Str); 1446 (CO-NH Str); 758 (C-Cl Str).<sup>1</sup>H NMR (DMSO, δ ppm): 7.68-8.34 (m, 6H, Ar-H), 7.7 (s, 1H, CH-Cl), 5.3 (s, 1H, CH-N), 4.8 (s, 1H, NH), 2.83 (s, 3H, OCH<sub>3</sub>), 3.48 (s, 4H, 4(OH)). MS (m/z): 365 (M<sup>+</sup>).

(3R, 4S)-3-Chloro-4-(2-chloro phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3c): Yield: 67 %, Colour: White, R<sub>f</sub> value: 0.72, M.p.; 217 °C. Anal. calcd. for C<sub>19</sub>H<sub>15</sub>CIN<sub>2</sub>O<sub>5</sub> (378): C, 53.39; H, 7.62; N, 6.28 %. Found: C, 52.12; H, 7.21; N, 6.58 %. IR (KBr in cm<sup>-1</sup>): 3614 (Ar-Oh Str); 2972 (Ar-CH Str); 1726 (C=O Str); 1598 (CONH Str); 1483 (CH-N Str); 752 (C-Cl Str).<sup>1</sup>H NMR (DMSO, δ ppm): 7.68-8.34 (m, 6H, Ar-H), 7.7 (s, 1H, CH-Cl), 5.5 (s, 1H, CH-N), 4.4 (s, 1H, NH), 3.9 (s, 3H, OCH<sub>3</sub>), 3.48 (s, 4H, 4(OH)). MS (m/z): 379 (M<sup>+</sup>).

(3R, 4S)-3-Chloro-4-(3-hydroxy phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3d): Yield: 63 %, Colour: Light brown, R<sub>f</sub> value: 0.56, M.p.; 243 °C. Anal. calcd. for C<sub>16</sub>H<sub>13</sub>CIN<sub>2</sub>O<sub>5</sub> (382): C, 52.06; H, 7.13; N, 6.39 %. Found: C, 52.12; H, 7.21; N, 6.58 %. IR ((KBr in cm<sup>-1</sup>): 3790 (Ar-Oh Str); 3330 (NH Str); 2972 (Ar-CH Str); 1726 (C=O Str); 1591 (CONH Str); 1488 (CH-N Str); 761 (C-Cl Str).<sup>1</sup>H NMR (DMSO, δ ppm): 7.12-8.48 (m, 6H, Ar-H), 7.7 (s, 1H, CH-Cl), 5.3 (s, 1H, CH-N), 4.8 (s, 1H, NH), 2.83 (s, 3H, 3(OH)). MS (m/z): 385 (M<sup>+</sup>).

(3R, 4S)-3-Chloro-4-(4-chloro phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3g): Yield: 63 %, Colour: Pale yellow, R<sub>f</sub> value: 0.41, M.p.; 198 °C. Anal. calcd. for C<sub>18</sub>H<sub>15</sub>CIN<sub>2</sub>O<sub>5</sub> (393): C, 50.83; H, 6.96; N, 9.36 %. Found: C, 50.55; H, 6.91; N, 9.29 %. IR (KBr in cm<sup>-1</sup>): 3790 (Ar-Oh Str); 3330 (NH Str); 2975 (Ar-CH Str); 1732 (C=O Str); 1592 (CONH Str); 1479 (CH-N Str); 761 (C-Cl Str).<sup>1</sup>H NMR (DMSO, δ ppm): 7.12-8.48 (m, 6H, Ar-H), 7.7 (s, 1H, CH-Cl), 5.3 (s, 1H, CH-N), 4.8 (s, 1H, NH), 2.83 (s, 3H, 3(OH)). MS (m/z): 383 (M<sup>+</sup>).

(3R, 4S)-3-Chloro-4-(2-nitro phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3h): Yield: 78 %, Colour: White, R<sub>f</sub> value: 0.49, M.p.; 215 °C. Anal. calcd. for C<sub>18</sub>H<sub>15</sub>CIN<sub>2</sub>O<sub>5</sub> (393): C, 50.83; H, 6.96; N, 9.36 %. Found: C, 50.55; H, 6.91; N, 9.29 %. IR (KBr in cm<sup>-1</sup>): 3790 (Ar-Oh Str); 3469 (NH Str); 2974 (Ar-CH Str); 1731 (C=O Str); 1598 (CONH Str); 1547 (C-NO<sub>2</sub> Str); 1483 (CH-N Str); 752 (C-Cl Str).<sup>1</sup>H NMR (DMSO, δ ppm): 7.58-8.58 (m, 6H, Ar-H), 7.7 (s, 1H, CH-Cl), 5.5 (s, 1H, CH-N), 4.4 (s, 1H, NH), 2.78 (s, 4H, 4(OH)).
ppm): 7.23-8.51 (m, 6H, Ar-H), 7.11 (s, 1H, CH-CI), 5.63 (s, 1H, CH-N), 4.49 (s, 1H, NH) 2.63 (s, 3H, 3(OH)). MS (m/z): 394 (M+1).

(3R, 4S)-3-Chloro-4-(3-nitro phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3l): Yield: 73%, Colour: White, Rf value: 0.75, M.p.: 224 °C. Anal. calcld. for C18H12ClN2O7(393): C, 50.83; H, 6.96; N, 9.36%. Found: C, 50.51; H, 6.83; N, 9.31%. IR (KBr in cm⁻¹): 3369 (Ar-OH Str); 3457 (NH Str); 1619 (C=O Str); 1589 (C-NO₂ Str). ¹H NMR (DMSO, δ ppm): 7.41-8.69 (m, 6H, Ar-H), 7.41 (s, 1H, CH-CI), 5.23 (s, 1H, CH-N), 4.52 (s, 1H, NH), 2.56 (s, 3H, 3(OH)). MS (m/z): 394 (M+1).

(3R, 4S)-3-Chloro-4-(4-dimethyl amino phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3o): Yield: 73%, Colour: White, Rf value: 0.66, M.p.; 231 °C. Anal. calcld. for C19H18ClN2O6(382): C, 52.06; H, 7.13; N, 6.45%. IR (KBr in cm⁻¹): 3769 (Ar-OH Str); 3408 (NH Str). ¹H NMR (DMSO, δ ppm): 7.33-8.78 (m, 6H, Ar-H), 7.32 (s, 1H, CH-CI), 5.57 (s, 1H, CH-CI), 4.81 (s, 1H, NH), 2.9 (s, 6H, N(CH₃)₂), 2.69 (s, 3H, 3(OH)). MS (m/z): 394 (M+1).

(3R, 4S)-3-Chloro-4-(4-methoxy phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3n): Yield: 66%, Colour: White, Rf value: 0.54, M.p.; 218 °C. Anal. calcld. for C19H18ClN2O6(378): C, 55.36; H, 7.90; N, 6.49%. Found: C, 55.32; H, 7.88; N, 6.45%. IR (KBr in cm⁻¹): 3769 (Ar-OH Str); 3402 (NH Str); 2974 (Ar-CH Str); 1755 (C-O Str); 1678 (CONH Str); 1472 (CH-N Str); 1301 (N-CH₃ Str); 769 (C-Cl Str). ¹H NMR (DMSO, δ ppm): 7.25-8.68 (m, 6H, Ar-H), 7.65 (s, 1H, CH-CI), 5.43 (s, 1H, CH-N), 4.41 (s, 1H, NH), 3.32 (s, 6H, (OCH₃)₂), 2.11 (s, 3H, 3(OH)). MS (m/z): 409 (M+1).

(3R, 4S)-3-Chloro-4-(3,4,5-trimethoxy phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3l): Yield: 69%, Colour: Pale brown, Rf value: 0.71, M.p.; 241 °C; Anal. calcld. for C19H19ClN₂O₆(438): C, 53.49; H, 7.75; N, 5.67%. Found: C, 53.51; H, 7.82; N, 5.65%. IR (KBr in cm⁻¹): 3740 (Ar-OH Str); 3298 (NH Str); 2973 (Ar-CH Str); 1724 (C=O Str); 1643 (CONH Str); 1488 (CH-N Str); 1258 (Ar-OCH₃ Str); 756 (C-Cl Str). ¹H NMR (DMSO, δ ppm): 8.21-8.78 (m, 4H, Ar-H), 7.51 (s, 1H, CH-CI), 5.68 (s, 1H, CH-N), 4.92 (s, 1H, NH), 3.60 (s, 9H, (OCH₃)₃), 2.47 (s, 3H, 3(OH)). MS (m/z): 439 (M+1).

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Trop J Pharm Res, April 2011;10 (2):225
**H NMR (DMSO, δ ppm):** 7.57-8.94 (m, 6H, Ar-H), 7.15 (s, 1H, CH-Cl), 5.12 (s, 1H, CH-N), 4.59 (s, 1H, NH), 2.45 (s, 3H, 3(OH)), MS (m/z): 383 (M⁺).

**Evaluation of antimicrobial activity**

The minimum inhibitory concentration (MIC) of the test compounds was determined by broth dilution method [19,20]. Each of the test compounds and the standard drugs were dissolved in DMSO at a concentration of 100 µg/ml. Further dilutions of the test compounds and standards in the test medium were prepared at concentrations of 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 µg/ml. The bacterial strains of *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were maintained on MHA (Muller-Hinton Agar) medium for 24 h at 37 °C while fungal strains of *Candida albicans* and *Aspergillus niger* were maintained on PDA (Potato Dextrose Agar) medium for 48 h at 25 °C. The bacterial and fungal suspensions were adjusted with sterile water to a concentration of approximately 103 CFU/ml and 104 CFU/ml, respectively. The test compounds and standard drugs, ciprofloxacin and ketoconazole, at various concentrations, were prepared in MHA medium for bacteria and PDA medium for fungi by two-fold serial dilution method and then inoculated and incubated for 24 h at 37 °C for bacteria and 48 h at 25 °C for fungi. The last tube with no growth of microorganism was recorded as the MIC, expressed in µg/ml.

**Evaluation of anti-tubercular activity**

Antitubercular activity was evaluated against *Mycobacterium tuberculosis* H37 Rv using Microplate alamar blue assay (MABA) method [21,22]. Antitubercular susceptibility test was performed in black, clear-bottomed, 96-well microplates (Packard Instrument Company, Meriden, Conn., USA) in order to minimize background fluorescence. Initial drug dilutions were prepared in dimethylsulfoxide and subsequent two-fold dilutions were performed in 0.1 ml of 7H9GC media in the microplates. An aliquot (100 µl) of 2000CFU/ml of *M. tuberculosis* H37 Rv were added to each well of 96-well microtitre plate containing test compounds. Three control well plates containing drug and medium, bacteria and medium, and medium only were also prepared. All microtitre plates were incubated at 37 °C for seven days. At day 7 of incubation, Alamar Blue dye solution (20 µl Alamar Blue solution and 12.5 ml of 20 % Tween 80) was added to all the wells and the plates re-incubated at 37 °C for 24 h. Fluorescence was measured in a Victor II multilabel fluorometer (Perkin Elmer Life Sciences Inc., Boston, MA, USA) and MIC was determined.

**Statistical anlaysis**

The antimicrobial study was a completely randomized study with three replications. Data were subjected to ANOVA using GraphPad Instat (Version 3.01, University of Kentucky, GTA-50429-259). A significant difference at \( p < 0.01 \) was set for the antimicrobial studies.

**RESULTS**

Physicochemical parameters of the synthesized compounds, including color, \( R_f \) value, and melting point are shown in Table 1 while antimicrobial and antitubercular results are listed in Table 2.

**Antimicrobial activity**

Compounds 3f, 3g, 3k and 3o were active, among the compounds in the series (3a-o), exhibiting antibacterial and antifungal activities as high as those of the reference standards - ciprofloxacin and ketoconazole, respectively. On the other hand, compounds 3a, 3c, 3h, 3i and 3j exhibited moderate activity.

**Antitubercular activity**

Compounds 3f, 3g, 3k and 3o showed MIC values equivalent to that of the reference standard, isoniazid. Compounds 3a, 3c, 3j
Table 1: Physicochemical parameters of the synthesized compounds

<table>
<thead>
<tr>
<th>Code</th>
<th>R</th>
<th>Colour</th>
<th>Mol. Wt</th>
<th>% Yield</th>
<th>Melting Pt.</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>H</td>
<td>Dull white</td>
<td>348.1</td>
<td>64</td>
<td>221</td>
<td>0.63</td>
</tr>
<tr>
<td>3b</td>
<td>2-OH</td>
<td>Pale yellow</td>
<td>364.7</td>
<td>68</td>
<td>198</td>
<td>0.78</td>
</tr>
<tr>
<td>3c</td>
<td>2-OH-3-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>White</td>
<td>394.7</td>
<td>68</td>
<td>217</td>
<td>0.72</td>
</tr>
<tr>
<td>3d</td>
<td>3-OH</td>
<td>White</td>
<td>364.7</td>
<td>62</td>
<td>206</td>
<td>0.69</td>
</tr>
<tr>
<td>3e</td>
<td>4-OH</td>
<td>Pale yellow</td>
<td>364.7</td>
<td>72</td>
<td>228</td>
<td>0.83</td>
</tr>
<tr>
<td>3f</td>
<td>2-Cl</td>
<td>Light Brown</td>
<td>382.1</td>
<td>71</td>
<td>243</td>
<td>0.56</td>
</tr>
<tr>
<td>3g</td>
<td>3-Cl</td>
<td>Pale yellow</td>
<td>382.1</td>
<td>63</td>
<td>211</td>
<td>0.41</td>
</tr>
<tr>
<td>3h</td>
<td>2-NO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>White</td>
<td>393.7</td>
<td>78</td>
<td>215</td>
<td>0.49</td>
</tr>
<tr>
<td>3i</td>
<td>3-NO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>White</td>
<td>393.7</td>
<td>66</td>
<td>224</td>
<td>0.75</td>
</tr>
<tr>
<td>3j</td>
<td>4-NO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>White</td>
<td>393.7</td>
<td>73</td>
<td>231</td>
<td>0.80</td>
</tr>
<tr>
<td>3k</td>
<td>4-N-(CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>White</td>
<td>391.8</td>
<td>61</td>
<td>234</td>
<td>0.66</td>
</tr>
<tr>
<td>3l</td>
<td>3,4,5-(OCH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Pale brown</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>23.4</td>
</tr>
<tr>
<td>3m</td>
<td>3,4,-(OCH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Pale brown</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>31.4</td>
</tr>
<tr>
<td>3n</td>
<td>4-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Pale yellow</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>2.8</td>
</tr>
<tr>
<td>3o</td>
<td>4-Cl</td>
<td>Pale yellow</td>
<td>382.1</td>
<td>72</td>
<td>204</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Table 2: Antimicrobial and antitubercular activities of compounds 3a-o

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Antibacterial activity</th>
<th>Antifungal activity</th>
<th>Antituberular activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BS</td>
<td>SA</td>
<td>SM</td>
<td>EC</td>
</tr>
<tr>
<td>3a</td>
<td>- H</td>
<td>12.5</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>3b</td>
<td>- 2-OH</td>
<td>25</td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>3d</td>
<td>- 3-OH</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>3e</td>
<td>- 4-OH</td>
<td>12.5</td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>3f</td>
<td>- 2-Cl</td>
<td>6.25</td>
<td>12.5</td>
<td>3.12</td>
</tr>
<tr>
<td>3g</td>
<td>- 3-Cl</td>
<td>1.56</td>
<td>1.56</td>
<td>3.12</td>
</tr>
<tr>
<td>3h</td>
<td>- 2-NO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>25</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>3i</td>
<td>- 3-NO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>6.25</td>
<td>12.5</td>
<td>3.12</td>
</tr>
<tr>
<td>3j</td>
<td>- 4-NO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>12.5</td>
<td>3.12</td>
<td>12.5</td>
</tr>
<tr>
<td>3k</td>
<td>- 4-N-(CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>3.12</td>
<td>1.56</td>
<td>1.56</td>
</tr>
<tr>
<td>3l</td>
<td>- 3,4,5-(OCH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>&gt;50</td>
<td>25</td>
<td>&gt;50</td>
</tr>
<tr>
<td>3m</td>
<td>- 3,4,-(OCH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
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<tr>
<td>3n</td>
<td>- 4-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>&gt;50</td>
<td>25</td>
<td>&lt;50</td>
</tr>
<tr>
<td>3o</td>
<td>- 4-Cl</td>
<td>3.12</td>
<td>6.25</td>
<td>3.12</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1.56</td>
<td>3.12</td>
<td>3.12</td>
<td>1.56</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Key: * Mean values (n = 3); BS - *Bacillus subtilis*; SA - *Staphylococcus aureus*; SM - *Streptococcus mutants*; EC - *Escherichia coli*; KP - *Klebsiella pneumoniae*; PA - *Pseudomonas aeruginosa*; CA - *Candida albicans*; AN - *Aspergillus niger*; MT - *Mycobacterium tuberculosis*; NA - Not Applicable
and 3n exhibited moderate activity, while other compounds were less active. The antitubercular activity results correlated well with those of antimicrobial activity.

DISCUSSION

The monocyclic 2-azetidinones, carbapenams and monobactams are active only against Gram-negative bacteria [23]. The synthesized compounds, however, showed about the same level of activity against both Gram-positive and Gram-negative bacteria. The results of antimicrobial activity and antitubercular activity revealed that compounds 3f, 3g, and 3o which have chloro substitution (electron withdrawing group) and 3k which has para dimethyl amino (bulker group) enhanced the activity of the azetidinones while the other groups, such as hydroxy, methoxy and nitro groups did not influence the activity. Among the chloro substituted compounds (3f, 3g, and 3o) compound 3g, which has a chloro group at meta position, exhibited the highest activity in the series. This suggests that electron withdrawing groups and bulker groups are responsible for the activity. It is interesting to note that some of the currently used antitubercular drugs, such as pyrazinamide, isoniazid and ethionamide, also possess electron withdrawing groups as its pharmacophore. Further studies are going on in our laboratory to establish the quantitative structure activity relationship (QSAR) of the series.

CONCLUSION

This study indicates that the antimicrobial and antitubercular activities of the synthesized compounds can be attributed to the presence of 2-Cl, 3-Cl, 4-Cl and 4-N-(CH₃)₂ in phenyl ring at the fourth position of the azetidinone nucleus. The findings of this work should be helpful to medicinal chemists involved in further drug development in this field.

ACKNOWLEDGEMENT

The authors are thankful to Dr R Shivakumar, Pro-Vice Chancellor, SRM University, Chennai, India and Dr KS Lakshmi, Dean, College of Pharmacy, SRM University, Chennai for making available the facilities used to carry out this work. We also express our profound gratitude to Dr. Ajaykumar for assistance in performing antitubercular tests.

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