

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 (^1H 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.

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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. ¹H NMR spectra were measured with a Bruker Spectrometer [400 MHz] in CDCl₃ using tetramethylsilane (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 ketaconazole were obtained from M/S Orchid Chemicals and Pharmaceuticals Limited, Chennai, India.

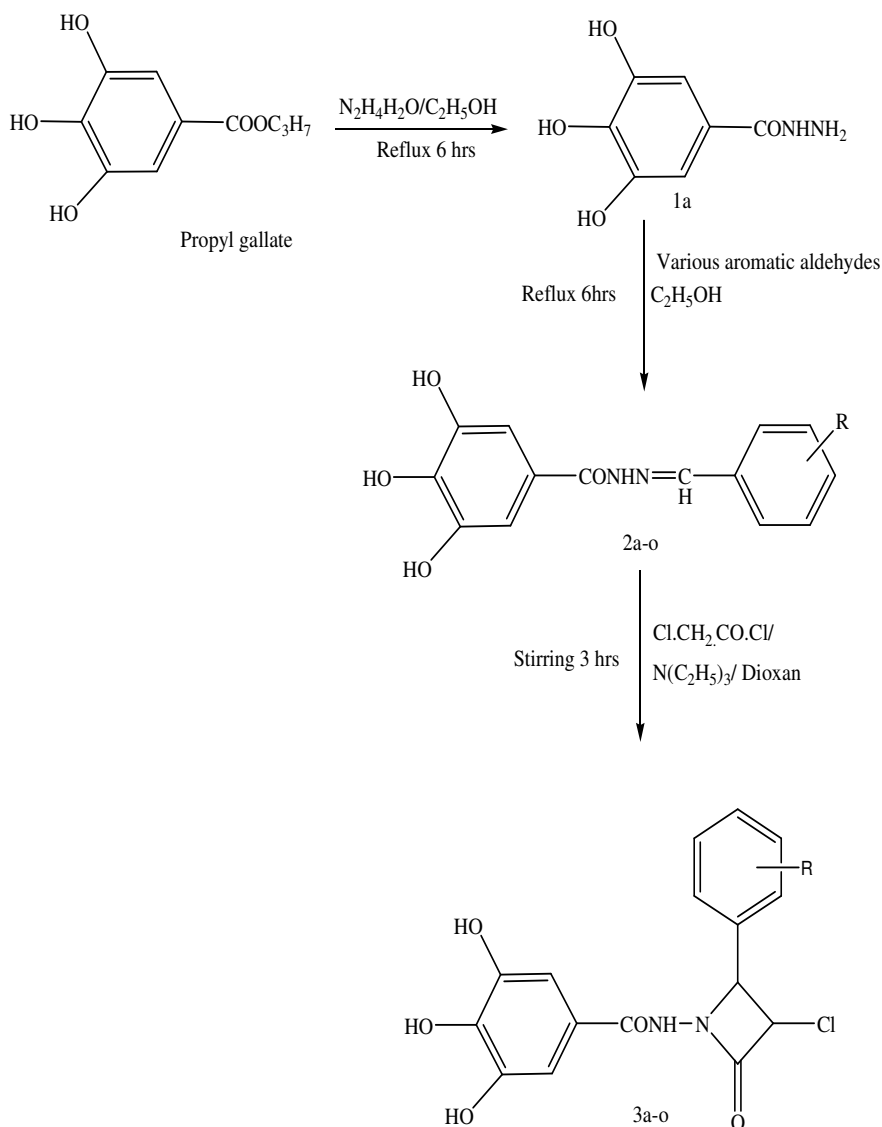


Fig 1: Synthesis of novel trihydroxybenzamido azetidin-2-one derivatives

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, M.p.; 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). ^1H NMR (DMSO, δ 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, M.p.; 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). ^1H NMR (DMSO, δ 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 white, R_f value: 0.61, M.p.; 171-172 °C. IR (KBr in cm^{-1}): 3331 (NH Str); 3310 (Ar-OH Str); 2994 (Ar-CH Str); 1678 (C=O Str); 1480 (C=N Str); 1444(CO-NH Str); 1264 (C-O-C Str). ^1H NMR (DMSO, δ 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-OCH₃), 3.48 (s, 4H, 4(OH)).

N'-(3-hydroxy) benzylidene galloyl hydrazide (2d): Yield: 79 % , Colour: Pale brown, R_f value: 0.61, M.p.; 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). ^1H NMR (DMSO, δ 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, M.p.; 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). ^1H NMR (DMSO, δ 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, M.p.; 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). ^1H NMR (DMSO, δ 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, M.p.; 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). ^1H NMR (DMSO, δ 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, 3(OH)).

N'-(2-nitro) benzylidene galloyl hydrazide (2h): Yield: 80 %, Colour: Pale brown, R_f value: 0.61, M.p.; 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-NO₂ Str); 1441 (C=N Str); 1421 (CO-NH Str). ^1H NMR (DMSO, δ 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, R_f value: 0.61, M.p.; 171-172°C. IR (KBr in cm^{-1}): 3400 (NH Str); 3312 (Ar-OH Str); 2971 (Ar-CH 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, R_f 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): 8.44-8.91 (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, R_f value: 0.61, M.p.; 175-178 °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): 8.31-9.33 (m, 6H, Ar-H), 8.42 (s, 1H, CH=N), 4.53 (s, 1H, NH), 2.21 (s, 3H, 3(OH)), 2.19 (s, 6H, N(CH₃)₂).

N'-(3,4,5-trimethoxy) benzylidene galloyl hydrazide (2l): Yield: 81 %, Colour: Pale yellow, R_f value: 0.61, M.p.; 171-173 °C. IR (KBr in cm^{-1}): 3397 (NH Str); 3313 (Ar-OH Str); 2977 (Ar-CH Str); 1687 (C=O Str); 1617 (C-O-C Str); 1480 (C=N Str); 1445 (CO-NH Str). ¹H NMR (DMSO, δ ppm): 8.18-8.85 (m, 4H, Ar-H), 8.28 (s, 1H, CH=N), 4.89 (s, 1H, NH), 3.62 (s, 9H, (OCH₃)₃), 2.71 (s, 3H, 3(OH)).

N'-(3,4-dimethoxy) benzylidene galloyl hydrazide (2m): Yield: 82 %, Colour: Pale brown, R_f 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, R_f 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, R_f 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): 8.39-8.95 (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-(phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3a): Yield: 70 %, Colour: Dull white, R_f value: 0.63, M.p.; 221°C. Anal. calcd. for C₁₆H₁₃ClN₂O₅ (348): C, 55.10; H, 3.76; N, 8.03 %. Found: C, 55.17; H, 3.68; N, 7.98 %. IR (KBr in cm^{-1}): 3613 (Ar-OH Str); 3467 (NH Str); 2977 (Ar-CH Str); 1744 (C=O Str); 1648 (CO-NH Str); 1474 (CH-N Str); 1446 (CO-NH Str); 759 (C-Cl Str). ¹H NMR (DMSO, δ ppm): 7.7 (s, 1H, CH-Cl), 7.08- 7.44 (m, 7H, Ar-H),

5.2 (s, 1H, CH-N), 4.15 (s, 1H, NH), 2.33 (s, 3H, 3(OH)). MS (m/z): 349 (M⁺).

(3R, 4S)-3-Chloro-4-(2-hydroxy phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3b): Yield: 73 %, Colour: Pale yellow, R_f value: 0.78, M.p.; 198 °C. Anal. calcd. for C₁₆H₁₃ClN₂O₆ (364): C, 54.34; H, 7.68; N, 6.67 %. Found: C, 54.32; H, 7.65; N, 6.65%. IR (KBr in cm⁻¹): 3613 (Ar-OH Str); 3467 (NH Str); 2977 (Ar-CH Str); 1755 (C=O Str); 1648 (CONH Str); 1474 (CH-N Str); 1446 (CO-NH Str); 757 (C-Cl Str). ¹H NMR (DMSO, δ ppm): 7.68-8.34 (m, 6H, Ar-H), 7.65 (s, 1H, CH-Cl), 5.43 (s, 1H, CH-N), 4.75 (s, 1H, NH), 2.76 (s, 4H, 4(OH)). MS (m/z): 365 (M⁺).

(3R, 4S)-3-Chloro-4-(2-hydroxy 3-methoxy phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3c): Yield: 67%, Colour: White, R_f value: 0.72, M.p.; 217 °C. Anal. calcd. for C₁₇H₁₅ClN₂O₇ (378): C, 53.39; H, 7.62; N, 6.23 %. Found: C, 53.37; H, 7.60; N, 6.28%. IR (KBr in cm⁻¹): 3614 (Ar-OH Str); 3458 (NH Str); 2985 (Ar-CH Str); 1743 (C=O Str); 1661 (CONH Str); 1482 (CH-N Str); 1446 (CO-NH Str); 1268 (C-O-C Str); 762 (C-Cl Str). ¹H NMR (DMSO, δ ppm): 7.12- 8.44 (m, 5H, Ar-H), 7.7 (s, 1H, CH-Cl), 5.5 (s, 1H, CH-N), 4.4 (s, 1H, NH), 3.9 (s, 3H, OCH₃), 3.48 (s, 4H, 4(OH)). MS (m/z): 379 (M⁺).

(3R, 4S)-3-Chloro-4-(3-hydroxy phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3d): Yield: 63 %, Colour: White, R_f value: 0.69, M.p.; 206 °C. Anal. calcd. for C₁₆H₁₃ClN₂O₆ (364): C, 54.34; H, 7.68; N, 6.67%. Found: C, 54.28; H, 7.59; N, 6.79%. IR (KBr in cm⁻¹): 3632 (Ar-OH Str); 3465 (NH Str); 2977 (Ar-CH Str); 1733 (C=O Str); 1667 (CONH Str); 1458 (CH-N Str); 1446 (CO-NH Str); 752 (C-Cl Str). ¹H NMR (DMSO, δ ppm): 7.52- 8.91 (m, 6H, Ar-H), 7.48 (s, 1H, CH-Cl), 5.5 (s, 1H, CH-N), 4.4 (s, 1H, NH), 3.31 (s, 4H, 4(OH)). MS (m/z): 365 (M⁺).

(3R, 4S)-3-Chloro-4-(4-hydroxy phenyl)-N-(3,4,5-trihydroxy benzamido)-2-

azetidinone (3e): Yield: 64 %, Colour: Pale yellow, R_f value: 0.83, M.p.; 228 °C. Anal. calcd. for C₁₆H₁₃ClN₂O₆ (364): C, 54.34; H, 7.68; N, 6.67%. Found: C, 54.38; H, 7.65; N, 6.69 %. IR (KBr in cm⁻¹): 3609 (Ar-OH Str); 3358 (NH Str); 2978 (Ar-CH Str); 1772 (C=O Str); 1653 (CONH Str); 1483 (CH-N Str); 758 (C-Cl Str). ¹H NMR (DMSO, δ ppm): 7.08- 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)). MS (m/z): 365 (M⁺).

(3R, 4S)-3-Chloro-4-(2-chloro phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3f): Yield: 71%, Colour: Light brown, R_f value: 0.56, M.p.; 243 °C. Anal. calcd. for C₁₆H₁₂Cl₂N₂O₅ (383): C, 52.06; H, 7.13; N, 6.39 %. Found: C, 52.12; H, 7.21; N, 6.58%. IR (KBr in cm⁻¹): 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). ¹H 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⁺).

(3R, 4S)-3-Chloro-4-(4-chloro phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3g): Yield: 63 %, Colour: Pale yellow, R_f value: 0.41, M.p.; 198 °C. Anal. calcd. for C₁₆H₁₂Cl₂N₂O₅(382): C, 52.06; H, 7.13; N, 6.39%. Found: C, 52.12; H, 7.21; N, 6.58 %. IR ((KBr in cm⁻¹): 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). ¹H 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⁺).

(3R, 4S)-3-Chloro-4-(2-nitro phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3h): Yield: 78 %, Colour: White, R_f value: 0.49, M.p.; 215 °C ; Anal. calcd. for C₁₆H₁₂ClN₃O₇ (393): C, 50.83; H, 6.96; N, 9.36 %. Found: C, 50.55; H, 6.91; N, 9.29%. IR (KBr in cm⁻¹): 3790 (Ar-OH Str); 3469 (NH Str); 2974 (Ar-CH Str); 1731 (C=O Str); 1598 (CONH Str); 1547 (C-NO₂ Str), 1483 (CH-N Str); 752 (C-Cl Str). ¹H NMR (DMSO, δ

ppm): 7.23-8.51 (m, 6H, Ar-H), 7.11 (s, 1H, CH-Cl), 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 (3i): **Yield:** 73%, **Colour:** White, **R_f value:** 0.75, **M.p.;** 224 °C. Anal. calcd. for $C_{16}H_{12}ClN_3O_7$ (393): C, 50.83; H, 6.96; N, 9.36 %. Found: C, 50.47; H, 6.83; N, 9.31 %. IR (KBr in cm^{-1}): 3369 (Ar-OH Str); 3457 (NH Str); 2978 (Ar-CH Str); 1745 (C=O Str); 1606 (CONH Str); 1533 (C-NO₂ Str); 1481 (CH-N Str); 759 (C-Cl Str). ¹H NMR (DMSO, δ ppm): 7.41-8.69 (m, 6H, Ar-H), 7.41 (s, 1H, CH-Cl), 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-nitro phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3j): **Yield:** 66%, **Colour:** White, **R_f value:** 0.80, **M.p.;** 231 °C. Anal.calcd. for $C_{16}H_{12}ClN_3O_7$ (393): C, 50.83; H, 6.96; N, 9.36 %. Found: C, 50.51; H, 6.67; N, 9.20 %. IR (KBr in cm^{-1}): 3330 (Ar-OH Str); 3462 (NH Str); 2976 (Ar-CH Str); 1745 (C=O Str); 1619 (CONH Str); 1548 (C-NO₂ Str); 1477 (CH-N Str); 751 (C-Cl Str). ¹H NMR (DMSO, δ ppm): 7.42-8.87 (m, 6H, Ar-H), 7.63 (s, 1H, CH-Cl), 5.55 (s, 1H, CH-N), 4.59 (s, 1H, NH), 2.34 (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 (3k): **Yield:** 61%, **Colour:** White, **R_f value:** 0.66, **M.p.;** 234 °C. Anal. calcd. for $C_{18}H_{18}ClN_3O_5$ (391): C, 56.43; H, 8.34; N, 9.40 %. Found: C, 56.31; H, 8.26; N, 9.35 %. IR (KBr in cm^{-1}): 3685 (Ar-OH Str); 3340 (NH Str); 2974 (Ar-CH Str); 1755 (C=O Str); 1678 (CONH Str); 1472 (CH-N Str); 1301 (N-CH₃ Str); 759 (C-Cl Str). ¹H NMR (DMSO, δ ppm): 7.33-8.78 (m, 6H, Ar-H), 7.32 (s, 1H, CH-Cl), 5.57 (s, 1H, CH-N), 4.81 (s, 1H, NH), 2.9 (s, 6H, N(CH₃)₂), 2.69 (s, 3H, 3(OH)). MS (m/z): 392 (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, **R_f value:** 0.71, **M.p.;** 241 °C; Anal.

calcd. for $C_{19}H_{19}ClN_2O_8$ (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}): 3470 (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-Cl), 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}).

(3R, 4S)-3-Chloro-4-(3,4-dimethoxy phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3m): **Yield:** 66%, **Colour:** Pale brown, **R_f value:** 0.81, **M.p.;** 203 °C; Anal. calcd. for $C_{18}H_{17}ClN_2O_7$ (408): C, 54.36; H, 7.82; N, 6.04 %. Found: C, 54.32; H, 7.78; N, 5.98%. IR (KBr in cm^{-1}): 3790 (Ar-OH Str); 3402 (NH Str); 2977 (Ar-CH Str); 1728 (C=O Str); 1648 (CONH Str); 1475 (CH-N Str); 1256 (Ar-OCH₃ Str); 764 (C-Cl Str). ¹H NMR (DMSO, δ ppm) 7.25-8.68 (m, 5H, Ar-H), 7.65 (s, 1H, CH-Cl), 5.43 (s, 1H, CH-N), 4.41 (s, 1H, NH), 3.32 (s, 6H, (OCH₃)₂), 2.21 (s, 3H, 3(OH)). MS (m/z): 409 (M^{+1}).

(3R, 4S)-3-Chloro-4-(4-methoxy phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3n): **Yield:** 73 %, **Colour:** Pale yellow, **R_f value:** 0.48, **M.p.;** 218 °C; Anal. calcd.for $C_{17}H_{15}ClN_2O_6$ (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); 2973 (Ar-CH Str); 1732 (C=O Str); 1637 (CONH Str); 1473 (CH-N Str); 1252 (Ar-OCH₃ Str); 750 (C-Cl Str). ¹H NMR (DMSO, δ ppm): 7.25-8.68(m, 6H, Ar-H), 7.52 (s, 1H, CH-Cl), 5.76 (s, 1H, CH-N), 4.89 (s, 1H, NH), 3.82 (s, 3H, OCH₃), 2.43 (s, 3H, 3(OH)). MS (m/z): 379 (M^{+1}).

(3R, 4S)-3-Chloro-4-(4-chloro phenyl)-N-(3,4,5-trihydroxy benzamido)-2-azetidinone (3o): **Yield:** 72%, **Colour:** Pale yellow, **R_f value:** 0.54, **M.p.;** 204 °C; Anal. calcd. for $C_{16}H_{12}Cl_2N_2O_5$ (382): C, 52.06; H, 7.13; N, 6.39 %. Found: C, 52.02; H, 7.11; N, 6.41%. IR (KBr in cm^{-1}): 3774 (Ar-OH Str); 3400 (NH Str); 2978 (Ar-CH Str); 1721 (C=O Str); 1625 (CONH Str); 1479 (CH-N Str); 764 (C-Cl Str).

^1H 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 $\mu\text{g/ml}$. 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 $\mu\text{g/ml}$. 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 $\mu\text{g/ml}$.

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 analysis

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

Code	R	Colour	Mol.Wt	% Yield	Melting Pt.	R _f .Value
3a	H	Dull white	348.1	64	221	0.63
3b	2-OH	Pale yellow	364.7	68	198	0.78
3c	2-OH-3-OCH ₃	White	394.7	68	217	0.72
3d	3-OH	White	364.7	62	206	0.69
3e	4-OH	Pale yellow	364.7	72	228	0.83
3f	2-Cl	Light Brown	382.1	71	243	0.56
3g	3-Cl	Pale yellow	382.1	63	211	0.41
3h	2-NO ₂	White	393.7	78	215	0.49
3i	3-NO ₂	White	393.7	66	224	0.75
3j	4-NO ₂	White	393.7	73	231	0.80
3k	4-N-(CH ₃) ₂	White	391.8	61	234	0.66
3l	3,4,5-(OCH ₃) ₃	Pale brown	438.8	69	241	0.71
3m	3,4,-(OCH ₃) ₂	Pale brown	408.7	66	203	0.81
3n	4-OCH ₃	Pale yellow	378.7	73	218	0.48
3o	4-Cl	Pale yellow	382.1	72	204	0.54

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

Compound	R	Antimicrobial study* (MIC, µg/ml)								
		Antibacterial activity						Antifungal activity		Antitubercular activity
		BS	SA	SM	EC	KP	PA	CA	AN	MT
3a	- H	12.5	25	25	12.5	25	12.5	12.5	6.25	2.8
3b	- 2-OH	25	12.5	25	25	12.5	25	25	25	50.3
3c	- 2-OH-3-OCH ₃	6.25	3.12	3.12	1.56	3.12	3.12	6.25	3.12	3.7
3d	- 3-OH	25	25	50	12.5	12.5	12.5	12.5	25	50.3
3e	- 4-OH	12.5	12.5	25	25	50	25	12.5	25	34.2
3f	- 2-Cl	6.25	12.5	3.12	6.25	3.12	6.25	3.12	1.56	0.76
3g	- 3-Cl	1.56	1.56	3.12	6.25	3.12	6.25	1.56	3.12	0.57
3h	- 2-NO ₂	25	12.5	12.5	25	25	12.5	25	25	17.1
3i	- 3-NO ₂	6.25	12.5	3.12	12.5	6.25	12.5	12.5	25	34.8
3j	- 4-NO ₂	12.5	3.12	12.5	12.5	12.5	3.12	6.25	12.5	1.2
3k	- 4-N-(CH ₃) ₂	3.12	3.12	1.56	1.56	3.12	3.12	1.56	3.12	0.62
3l	- 3,4,5-(OCH ₃) ₃	>50	25	>50	25	>50	25	25	25	23.4
3m	- 3,4,-(OCH ₃) ₂	>50	>50	>50	25	25	>50	25	25	31.4
3n	- 4-OCH ₃	>50	25	25	>50	>50	>50	>50	>50	2.8
3o	- 4-Cl	3.12	6.25	3.12	6.25	3.12	12.5	6.25	3.12	0.83
Ciprofloxacin		1.56	3.12	3.12	1.56	3.12	1.56	NA	NA	NA
Ketoconazole		NA	NA	NA	NA	NA	NA	1.56	3.12	NA
Isoniazid		NA	NA	NA	NA	NA	NA	NA	NA	0.56

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 (bulkier 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 bulkier 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.

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