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SYNTHESIS AND ANTITUBERCULAR ACTIVITY OF BERBERINE DERIVATIVES

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*The isoquinoline alkaloid berberine (**1**) was isolated from the roots of* Berberis aristata *and its new, 13-benzyl (**3–6**), 13-allyl (7, **8**), 8-(2-oxopropyl) (**2**), and 9-hydroxy (**9**) derivatives have been synthesized under mild conditions with good yield. The structures of the new derivatives were confirmed by spectroscopic (UV, IR, NMR, and MS) analysis. The antitubercular activity of the derivatives against* Mycobacterium tuberculosis *$H_{37}Rv$ was studied (microdilution assay) and compared with rifampicin as standard drug. The results demonstrated that the 4-chlorobenzyl (**4**), 2,4-dichlorobenzyl (**5**), 4-fluorobenzyl (**6**), and 3′,3′-dimethylallyl (**8**) derivatives exhibited (MIC, 4–8 μg/mL) 2–4 fold more activity than berberine (MIC, 16 μg/mL), which is probably due to the 13-benzyl and allyl substitution in the molecule.*

Keywords: *Mycobacterium tuberculosis* $H_{37}Rv$, antitubercular, berberine, *Berberis aristata,* alkaloid, 13-substituted derivative.

Tuberculosis is the leading cause of infectious disease mortality in the world [1].

Despite the efforts of researchers in design, synthesis, and development of new antitubercular regimens [2], no new drug against tuberculosis has been developed since 1960. All the above facts intensified the need to develop new, novel, safe, and more efficient drugs with unique and divergent structure and a novel mechanism of action on different molecular targets aimed at a better understanding of antimycobacterial resistance.

Berberine, an isoquinoline alkaloid, possesses a variety of pharmacological activities such as antimicrobial, antileukemic, antiulcerous, and enzyme inhibiting [3, 4], anti-inflammatory [5], anti-diarrhea [6], glucose-lowering [7], cholesterol-lowering [8], neuroprotective [9], antidepressant [10], Alzheimer′s disease-ameliorating [11], etc. It has been reported that berberine analogues exhibit activity on LDLR [12]. Berberine and its derivatives showed cytotoxicity against HeLa, SVKO3, Hep-2 [13], and antileishmaniasis activity [14].

Scientific and technological advances in recent decades have made it possible to understand the pathways involved in inhibition of *M. tuberculosis*. This knowledge permits the development of new drugs synthesized from plant-derived active principles and their synthetic analogues that are able to interfere with these pathways and potentially act as antitubercular agents [15]. Berberine has been reported to exhibit good activity against *M. intracellulare* and weak activity against *M. smegmatis* and *M. tuberculosis* [16], whereas it is more active against *M. smegmatis* than *M. tuberculosis* (25 mg/L) [17]. But its derivatives have not been explored for activity. Hence, it is expected that berberine derivatives might show better antitubercular activity than berberine.

In continuation of our research to identify potent lead compounds against tuberculosis, we have directed our efforts towards generating new chemical entities that can be effective antitubercular agents with low toxicity. In the present study, the synthesis of a series of new 13-benzyl and allyl berberine bromides and other derivatives of berberine and their antitubercular activity against *M. tuberculosis* $H_{37}Rv$ was carried out.

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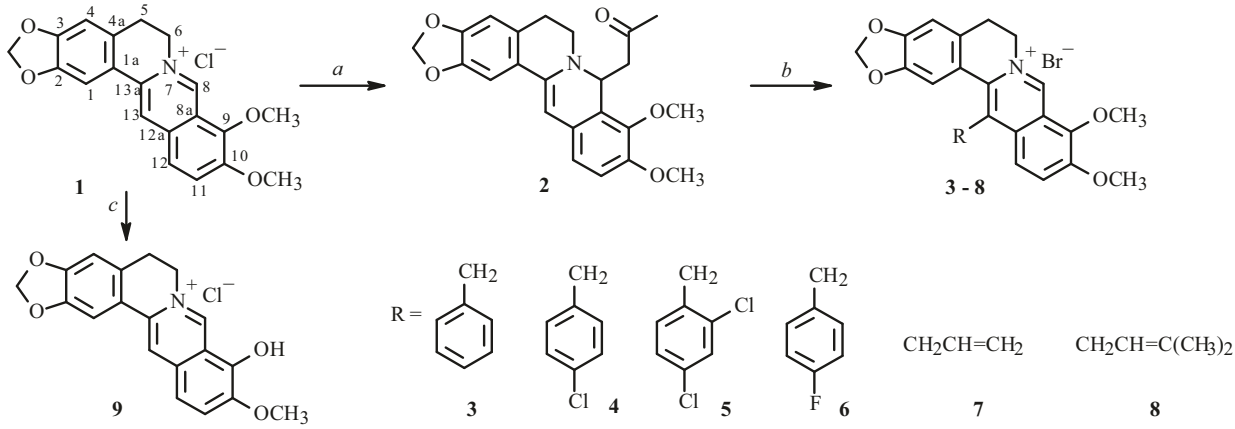
TABLE 1. Antitubercular Activity of Berberine and Its Derivatives

Compound	MIC, μg/mL	Compound	MIC, μg/mL
1	16	**6**	8
2	16	**7**	> 16
3	16	**8**	4
4	4	**9**	> 16
5	8	Rifampicin	0.03

MIC: Minimum inhibitory concentration.



a. acetone, 5N NaOH, stirring, 3 h, r.t.; *b*. RBr, CH_3CN, NaI, reflux, 80°C, 2 h; *c*. DMF, reflux, 190°C, 2 h

Scheme 1

Berberine (**1**) was isolated from *Berberis aristata* root bark extract as yellow crystals (2.5%). Various 13-benzyl and allyl substituted analogues (**3–8**), 8-(2-oxopropyl)berberine, and 9-hydroxyberberine were synthesized (Scheme 1).

Compound **2**, 8-(2-oxopropyl)berberine was prepared by the reaction of berberine with acetone at room temperature. Analogues **3–8** were synthesized by the reaction of compound **2** in acetonitrile and sodium iodide with the corresponding benzyl and allyl bromides at 80°C for 2 h. The structural elucidation was based on spectral analysis such as UV, IR, NMR, and mass data, as well as comparison with those of earlier reported data [4].

The NMR spectra of 13-benzylberberine bromide (**3**) showed the absence of the H-13 proton and additional peaks of the benzyl group as doublet at δ 7.17 and two triplets at δ 7.29 and 7.35, indicating substitution at the C-13 position. The 4-chlorobenzyl derivative of berberine, **4**, was identified by the presence of two doublets of the benzyl ring at δ 7.16 and 7.36. In the case of the 2,4-dichlorobenzyl derivative (**5**), NMR spectra showed the presence of one singlet and two doublets at δ 7.69, 6.85, and 7.23, respectively, and the absence of C-13 proton. The fluoro compound (**6**) was identified by the presence of two triplets and one doublet in the aromatic region at δ 7.18, 7.09, and 7.02, respectively, along with the absence of the C-13 proton. The chemical shifts for allyl derivative (**7**) were obtained as an allylic multiplet at 6.5 ppm and a vinylic doublet at δ 5.4 ppm. The 3′,3′-dimethylallyl derivative (**8**) was identified by the presence of methyl protons present at δ 1.8 with integration for six protons, and the absence of the C-13 proton in the spectra. The ^{1}H NMR spectra of 9-hydroxyberberine showed the absence of one of the methoxy groups. All the structures were confirmed by the mass spectra. Of the eight derivatives synthesized, compounds **4–8** are new derivatives of berberine.

Antitubercular Activity. All the synthesized derivatives were screened for their antitubercular activity against the drug-resistant *M. tuberculosis* $H_{37}Rv$, and MIC were calculated using microdilution assay (Table 1).

The results reveal that all derivatives except 13-allyl- and 9-hydroxyberberine chloride exhibited good activity (4–16 μg/mL) against *M. tuberculosis* $H_{37}Rv$. From the comparative studies, it is possible to draw some structure–activity relationships. Introduction of a substituted benzyl or a 3′,3′-dimethylallyl group at C-13 enhanced the activity. The derivatives 13-(4-chlorobenzyl)-berberine bromide (**4**) and 13-(3′,3′-dimethyl allyl)-berberine bromide (**8**) exhibited fourfold greater activity (MIC 4 μg/mL) than the parent pharmacophore, while 13-(2,4-dichlorobenzyl)-berberine bromide (**5**) and 13-(4-flourobenzyl)-berberine bromide (**6**) exhibited twofold (MIC 8 μg/mL) greater activity. The unsubstituted benzyl compound, 13-(benzyl)-berberine

bromide, exhibited the same activity as berberine (16 μg/mL). The weak activity of the 9-hydroxy derivative can be attributed to the absence of the methoxy group, which is essential for the activity. The presence of two methyl groups in 13-(3′,3′-dimethylallyl)-berberine bromide (**8**) increased the activity. The synthesized compounds were found to be nontoxic up to 50 μg/mL on rat macrophages.

In conclusion, a series of 13-benzyl (substituted) and allyl derivatives were synthesized from the natural scaffold berberine and evaluated for their antitubercular activity. Four of the derivatives exhibited four- and twofold greater activity than the natural scaffold, and the presence of the methoxy group in both is needed for the activity. The study suggests that promising moieties of berberine and/or the active derivatives can be developed as new antitubercular agents by further structural modification.

EXPERIMENTAL

Melting points were determined on a Veego VMP-DS apparatus and are uncorrected. The λ_{max} values were obtained using a Shimadzu UV-1800 UV spectrophotometer. IR spectra (KBr disc) were recorded on a Buck Scientific 500 spectrometer. ^{1}H NMR (500 MHz) spectra were obtained on a Bruker-500 NMR spectrometer. Chemical shifts are expressed in (δ) ppm relative to the internal standard TMS. Deuterated solvent for NMR spectra was CD_3OD. The ESI mass spectra were obtained using a PerkinElmer mass spectrometer. All chemicals were purchased from Sigma Aldrich and Merck. All solvents used were of analytical grade and were distilled prior to use. Thin layer chromatography was done on Merck Silica Gel 60 F-254 alumina sheets, and purification of compounds was done by column chromatography using Qualigen Silica Gel 60 (60–120 mesh).

Extraction and Isolation. The root bark of *Berberis aristata* was shade dried, powdered (100 g), and extracted by cold percolation with 2.5% acetic acid for 12 h. It was then filtered and concentrated. Concentrated hydrochloric acid was added untill precipitation occurred and the whole kept at 4°C. The yellow precipitate was separated by filtration and dried under vacuum. The residue was then crystallized from water and methanol (7:3) to afford compound **1**.

Berberine Chloride (1). Obtained as yellow crystals, yield 2.5%, mp 194°C (uncorrected) [18]. UV (MeOH, λ_{max}, nm): 229, 265, 350, 435. IR (KBr, ν, cm^{-1}): 3399, 2930, 2839, 1592, 1564 (Ar), 1483, 1442, 1354. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm): 3.26 (2H, t, H-5), 4.10 (3H, s, OCH_3), 4.20 (3H, s, OCH_3), 4.92 (2H, t, H-6), 6.10 (2H, s, OCH_2O), 6.96 (1H, s, H-4), 7.66 (1H, s, H-1), 8.00 (1H, d, H-12), 8.11 (1H, d, H-11), 8.70 (1H, s, H-13), 9.76 (1H, s, H-8). ESI-MS *m/z* : 336.1 [M^+], 338.2 [M + 2].

Synthesis of 8-Hydro-8-(2-oxopropyl)-berberine (2). Berberine chloride (0.5 g, 1.3 mmol) was dissolved in 5 N NaOH (2.3 mL), and acetone (0.5 mL) was added dropwise with stirring. The reaction was continued for 2 h. The reaction mixture was filtered and washed with 80% methanol to give 8-acetonylberberine.

Obtained as dark yellow crystals, yield 60%, mp 170°C. UV (MeOH, λ_{max}, nm): 230, 265, 350, 433. IR (KBr, ν, cm^{-1}): 2935, 2837, 1703 (C=O), 1595 (Ar), 1487, 1448, 1350. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm): 2.11 (3H, s, $COCH_3$), 2.80 (2H, d, CH_2), 3.77 (2H, t, H-5), 3.77 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 4.01 (1H, t, H-8), 4.92 (2H, t, H-6), 6.10 (2H, s, OCH_2O), 7.13 (1H, s, H-1), 6.75 (1H, d, H-12), 6.83 (1H, d, H-11), 7.46 (1H, s, H-13). ESI-MS *m/z* 394.3 [M + 1]$^+$.

General Procedure for Preparation of 3–8. Compound **2** (0.2 g, 0.51 mmol) was dissolved in acetonitrile and sodium iodide, and the appropriate benzyl/allyl bromides (1 mmol) were added and heated at 80°C for 2 h. The reaction was monitored by TLC. It was dried under reduced pressure. The residue was purified by silica gel column chromatography using appropriate eluent ($CHCl_3$ and methanol). The fractions containing target compounds were crystallized from methanol and hexane to afford compounds **3–8**.

13-(Benzyl)-berberine Bromide (3). Obtained as dark yellow crystals, yield 52%, mp 220°C. UV (MeOH, λ_{max}, nm): 208, 231, 267, 344, 423. IR (KBr, ν, cm^{-1}): 2927, 2862, 1458 (Ar), 1352, 1261. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm): 3.25 (2H, t, H-5), 4.06 (3H, s, OCH_3), 4.23 (3H, s, OCH_3), 6.10 (2H, s, OCH_2O), 7.02 (1H, s, H-4), 7.05 (1H, s, H-1), 7.17 (2H, t, H-2′, 6′), 7.29 (2H, t, H-3′, 5′), 7.37 (1H, t, H-4′), 7.82 (1H, d, H-12), 8.00 (1H, d, H-11), 9.91 (1H, s, H-8). ESI-MS *m/z*: 426.3 [M^+], 428.2 [M + 2].

13-(4-Chlorobenzyl)-berberine Bromide (4). Obtained as dark yellow crystals, yield 40%, mp 250°C. UV (MeOH, λ_{max}, nm): 224, 266, 344, 425. IR (KBr, ν, cm^{-1}): 2925, 2867, 1531, 1456 (Ar), 1361, 1267. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm): 3.19 (2H, t, H-5), 4.06 (3H, s, OCH_3), 4.24 (3H, s, OCH_3), 6.02 (2H, s, OCH_2O), 6.99 (1H, s, H-4), 7.03 (1H, s, H-1), 7.17 (2H, d, H-2′, 6′), 7.36 (2H, d, H-3′, 5′), 7.82 (1H, d, H-12), 8.00 (1H, d, H-11), 9.92 (1H, s, H-8). ESI-MS *m/z*: 460.9 [M^+], 462.0 [M + 2], 464 [M + 4].

13-(2,4-Dichlorobenzyl)-berberine Bromide (5). Obtained as dark yellow crystals, yield 36%, mp 259°C. UV (MeOH, λ_{max}, nm): 206, 229, 267, 344, 423. IR (KBr, ν, cm^{-1}): 2977, 2897, 1604, 1504 (Ar), 1454, 1379, 1265. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm): 3.19 (2H, t, H-5), 4.07 (3H, s, OCH_3), 4.24 (3H, s, OCH_3), 6.03 (2H, s, OCH_2O), 6.79 (1H, s, H-4), 6.85 (1H, d, H-6′), 7.04 (1H, s, H-1), 7.23 (1H, d, H-5′), 7.69 (1H, s, H-3′), 7.68 (1H, d, H-12), 8.01 (1H, d, H-11), 9.95 (1H, s, H-8). ESI-MS *m/z*: 494.1 [M^+], 496.1 [M + 2], 498.0 [M + 4], 500.0 [M + 6].

13-(4-Fluorobenzyl)-berberine Bromide (6). Obtained as dark yellow crystals, yield 39%, mp 243°C. UV (MeOH, λ_{max}, nm): 205, 231, 266, 344, 423. IR (KBr, ν, cm^{-1}): 2943, 2873, 1595, 1452 (Ar), 1379. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm): 3.18 (2H, t, H-5), 4.06 (3H, s, OCH_3), 4.23 (3H, s, OCH_3), 6.02 (2H, s, OCH_2O), 7.01 (1H, s, H-4), 7.03 (1H, s, H-1), 7.10 (2H, d, H-2′, 6′), 7.18 (2H, H-3′, 5′), 7.80 (1H, d, H-12), 8.00 (1H, d, H-11), 9.92 (1H, s, H-8). ESI-MS *m/z*: 444.1 [M^+], 446.1 [M + 2].

13-(Allyl)-berberine Bromide (7). Obtained as dark yellow crystals, yield 44%, mp 209°C. UV (MeOH, λ_{max}, nm): 205, 229, 265, 343, 416. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm): 3.17 (2H, t, H-5), 4.05 (3H, s, OCH_3), 4.11 (3H, s, OCH_3), 4.92 (2H, d, H-3′), 5.42 (2H, d, H-1′), 6.10 (2H, s, OCH_2O), 6.45 (1H, m, H-2′), 7.02 (1H, s, H-4), 7.47 (1H, s, H-1), 8.02 (1H, d, H-12), 8.11 (1H, d, H-11), 9.85 (1H, s, H-8). ESI-MS *m/z*: 376.3 [M^+], 378.1 [M + 2].

13-(3′,3′-Dimethylallyl)-berberine Bromide (8). Obtained as dark yellow crystals, yield 48%, mp 217°C. UV (MeOH, λ_{max}, nm): 205, 230, 265, 343, 421. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm): 1.85 (6H, s, CH_3), 3.11 (2H, t, H-5), 4.00 (2H, d, H-1′), 4.16 (3H, s, OCH_3), 4.21 (3H, s, OCH_3), 4.81 (1H, t, H-2′), 6.10 (2H, s, OCH_2O), 7.02 (1H, s, H-4), 7.31 (1H, s, H-1), 8.05 (1H, d, H-12), 8.16 (1H, d, H-11), 9.81 (1H, s, H-8). ESI-MS *m/z*: 404.4 [M^+], 406.2 [M + 2].

Synthesis of 9-Hydroxyberberine Chloride (9). A mixture of compound **1** (0.2 g, 0.54 mmol) and dimethyl formamide (0.5 mL) was refluxed for 2 h at 190°C. The reaction mixture was purified on a silica gel (60–120 mesh) column eluted with solvents of increasing polarity from chloroform to methanol. The target compound was further crystallized from methanol and hexane.

Obtained as dark yellow crystals, yield 65%, mp 269°C. UV (MeOH, λ_{max}, nm): 209, 238, 276, 333, 389, 510. IR (KBr, ν, cm^{-1}): 3396 (OH), 2921, 2852, 1627, 1510 (Ar), 1362, 1220. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm): 3.14 (2H, t, H-5), 3.9 (3H, s, OCH_3), 4.61 (2H, t, H-6), 6.04 (2H, s, OCH_2O), 6.86 (1H, s, H-4), 6.99 (1H, d, H-12), 7.58 (1H, s, H-1), 7.59 (1H, d, H-11), 8.12 (1H, s, H-13), 9.32 (1H, s, H-8). ESI-MS *m/z*: 322.1 [M^+], 324.5 [M + 2].

Antituberculosis Assay. The antitubercular activity of the derivatives along with berberine was evaluated against standard sensitive strain *Mycobacterium tuberculosis* $H_{37}Rv$. The minimum inhibitory concentration (MIC) was determined using broth microdilution assay [19, 20]. The *M. tuberculosis* $H_{37}Rv$ was grown to mid-log phase (10–12 days) at 37°C with shaking in sterile Middlebrook 7H9 broth supplemented with 10% ADC (BD Biosciences, USA). The turbidity of the culture was adjusted to be equivalent to 1 McFarland turbidity standard (~1×10^7 CFU/mL), which was further diluted 1:10 in the above-mentioned media. Stock solutions (1 mg/mL) of the compounds were prepared in DMSO-d_6, and nine 2-fold serial dilutions of the compounds were prepared in 100 μL volume of the above-mentioned media in 96-well U bottom microtiter plates. A 100 μL volume of the diluted inoculum was added to each well of the plate, resulting in a final inoculum of 5×10^5 CFU/mL. The final concentrations of the compounds after the addition of the inoculums were 0.12–32 μg/mL. Rifampicin in the concentration range 0.06–16 μg/mL was used as control drug. Periphery wells of the plate were filled with sterile distilled water to prevent evaporation of media in the wells. The plates were incubated at 37°C under 5% CO_2 for 3 weeks. Inhibition of growth was determined both by visual examination and with a spectrophotometer at OD 600. The lowest concentration of the compound showing no turbidity was recorded as MIC.

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