

## Synthesis of Putrescine Bisamides as Antimicrobial and Anti-Inflammatory Agents

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### Abstract

A new naturally occurring  $N_1$ ,  $N_6$ -dihydrocinnamyl putrescine bisamide, JBIR-94, along with nine structural analogs and a series of substituted phenyl and alkyl putrescine bisamides have been synthesized from putrescine and appropriately substituted carboxylic acids, through carboxylic acid chlorides. Antimicrobial, 5-Lipoxygenase enzyme inhibitory and antioxidant studies were performed for all synthesized compounds. Dihydrocinnamyl series of putrescine bisamides (4a-4i) showed good bioactivities compared to substituted phenyl (6a-6g) and dialkyl (6h-6j) series of compounds. Among all compounds, 4h (methylenedioxy analog) and 4a (JBIR-94) showed good antimicrobial, anti-inflammatory and antioxidant activities.

**Keywords:** JBIR-94; Putrescine bisamide; Antimicrobial; Antioxidant; 5-LOX; Docking

### Introduction

Putrescine bisamides are one of the subclasses of naturally occurring polyamides [1]. During the last two decades several symmetrical and unsymmetrical putrescine bisamides have been isolated from *Aglaia* [2-4], *Liberica* [5], and *Carydalis* [6], species. These compounds have been reported to possess various biological activities including cytotoxicity [7], anti-inflammatory [8], antioxidant [9,10], insecticidal [11,12], and antiviral activity. Putrescine derivatives have been hypothesized to be one of the precursors in bio-synthesis of rocaglamides, which have displayed pronounced anti proliferative activity against Human cancer cell [10,13], and exhibit strong insecticidal activity against *Spodoptera littoralis* [13]. The insecticidal activity of rocaglamides is comparable to the potency of azadirachtin [13]. Synthesis of these compounds is usually based on the condensation of putrescine with carboxylic acid chlorides in the presence of bases, or with carboxylic acids in the presence of coupling reagents such as 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC),  $N,N'$ -Dicyclohexylcarbodiimide (DCC) and (Benzotriazol-1-yloxy)tris(dimethylamino) phosphonium hexafluorophosphate (BOP). Recently, a new  $N_1$ ,  $N_6$ -dihydrocinnamyl putrescine bisamide, JBIR-94, has been isolated from broth culture of *Streptomyces* (strain R56-07), and reported to have antioxidant activities [9]

### Experimental Section

#### General

Melting points were recorded on a Mel-Temp melting point apparatus, in open capillaries and are uncorrected. <sup>1</sup>H NMR (400 MHz), <sup>13</sup>C NMR (100 MHz) spectra were recorded on a Bruker AMX 400 MHz NMR spectrometer using TMS as internal standard and the values for chemical shifts ( $\delta$ ) being given in ppm and coupling constants ( $J$ ) in Hertz (Hz). Mass spectra were recorded on an Agilent 1100 LC/MSD. Acme silica gel G and silica gel (100-200 mesh) were used for analytical TLC and column chromatography, respectively. Other chemicals were purchased from Sigma Aldrich and used without further purification.

**General experimental procedure for synthesis of 3:** A mixture of carboxylic acid (6.58 mmol) and thionyl chloride (13.1 mmol) was refluxed at 80°C for 1 h. After 1 h excess of SOCl<sub>2</sub> was removed under reduced pressure. Triethylamine (14.2 mmol) and putrescine (3.3

mmol) dissolved in DCM was added to the above reaction mixture at 0°C and warm to room temperature for 1h. Then the solution was diluted with CHCl<sub>3</sub> and washed consequently with 2N HCl, saturated aq NaHCO<sub>3</sub> and brine solution. The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub> and the solvent was removed under vacuum. The residue was suspended in ethyl acetate filtered off and washed with cold ethyl acetate and dried to obtain the pure product. It was directly used in the next step.

**General experimental procedure for synthesis of 4:** The compound (0.5 gm) (3) was dissolved in THF, and 10% palladium on carbon (5 mg, 10%) was added. The mixture was stirred under hydrogen balloon pressure at ambient temperature for 2 h. The reaction mixture was filtered through celite bed and washed with methanol. Methanol was evaporated under reduced pressure and the resultant solid was recrystallized in cold ethyl acetate to afford title compound.

**General experimental procedure for synthesis of 6:** Putrescine (3.3 mmol) and triethylamine (14.3 mmol) were added to the solution of acid chloride (7.6 mmol) in CH<sub>2</sub>Cl<sub>2</sub> at 0°C. The reaction mixture was then allowed to stir at room temperature for 0.5 h. After the completion of the reaction mixture was basicified with saturated aq NaHCO<sub>3</sub> solution and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure to afford 6. This was purified by recrystallization with hexane.

#### Antimicrobial assay

Antimicrobial assay performed on human pathogenic bacteria and fungi. *Salmonella typhi*, *Vibrio cholerae*, *Shigella dysenteriae*, *Enterococcus faecalis* are gastrointestinal pathogenic bacteria, which

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are clinical isolates collected from King George Govt. Hospital, Visakhapatnam, India. *Staphylococcus aureus* (NCIM 3021) culture was purchased at NCL, Pune, India. *Candida albicans* is a dermatophytic fungus collected from K. Ramamurthy memorial hospital, Ravivalsa, India. Zone of inhibitions were determined using agar well diffusion method and minimum inhibitory concentration (MIC) was done by broth dilution assay. Microbial broth cultures (Mueller Hinton broth for bacteria, Sabouraud Dextrose broth for fungi) were adjusted to an absorbance of 0.6 (Optical Density at 620 nm) in Spectrophotometer according to CLSI guidelines. These cultures were used as Inoculums for antimicrobial study. The agar plates were prepared by pour plate method using 20 ml of sterilized agar medium (MH agar for bacteria, SD agar for fungi). The sterile agar medium was cooled to 45°C and mixed thoroughly with 1ml of growth culture of concerned test organism (inoculum) and then poured into the sterile petri dishes and allowed to solidify. Wells of 6 mm size were made with sterile cork borer and test compounds were added. The agar plates were incubated at for 4 days at 28°C for fungi while 24 hours at 37°C for bacteria. Zone of inhibitions were measured by Himedia milli meter zone reader. Minimum Inhibitory Concentration (MIC) was performed on both media (10 ml) containing 1000-1 µg/ml of test compound prepared by 10 fold dilution. 0.1 ml of culture inoculums was added. The MIC was determined at the concentration of compound that causes nil absorbance (no growth) in the spectrophotometer at 620 nm. All the experiments were conducted according to Clinical Laboratory Standard Institute. Ciprofloxacin (for bacteria) and Griseofulvin (for fungi) were used as positive control. DMSO used as negative control [14-18].

### 5-Lipoxygenase (5-LOX) inhibitory assay

5-LOX from potato tubers was purified and assayed as per the method described by Reddanna et al. [18] The assay mixture contained 80 mM linoleic acid and sufficient amount of potato 5-Lipoxygenase enzyme in 50 mM phosphate buffer (pH 6.3). The reaction was initiated by the addition of enzyme buffer mix to substrate (linoleic acid) and the enzyme activity was monitored by an increase in absorbance at 234 nm for 120 seconds using UV Kinetic mode on Varian Cary-50 UV-VIS spectrophotometer. In the inhibition studies the activities were measured by incubating various concentration of compound with enzyme buffer mix for two minutes before adding the substrate. The assay was performed in triplicate. Percentage of inhibition was calculated by change in absorbance of test with that of control enzyme activity. Nordihydroguaiaretic acid (NDGA) was used as positive control [19-21].

### Molecular docking studies

5-Lipoxygenase (PDB ID 308Y) X-Ray crystal structure was obtained from Protein Data Bank and used in docking studies. Co-crystallized ligands and water molecules are removed from target protein using Argus lab. Ligands are prepared using Chemoffice (Cambridge). Energy minimization was done using molecular mechanics. The minimized was executed until root mean square value reached smaller than 0.001 Kcal/mol. Such energy minimized ligands and receptor used for docking studies using GEMDOCK (Generic Evolutionary Method for molecular DOCKing) is a generic evolutionary method with an empirical scoring function for the protein-ligand docking, which is a problem of paramount importance in structure-based drug design, combines both continuous and discrete search mechanisms. A population size of 300 with 70 generations and 3 solutions were used in docking accuracy setting. PyMol is used for better visualization of interactions [22-24].

### DPPH radical scavenging activity

DPPH (1, 1-diphenyl -2-picryl -hydrazyl) radical scavenging activity of the compounds was determined by the method of Lamaison et al., [19] which depends on scavenging of coloured free radical (DPPH) in methanol solution by test compound. The reaction mixture contains DPPH and compound in a final concentration in 3ml. Absorption of DPPH at its absorption maximum 516 nm is inversely proportional to scavenging activity of compound. The activity was expressed as inhibitory concentration 50 (IC<sub>50</sub>) i.e. the concentration of test compound required to give 50% reduction in absorbance of test solution compared to that of blank solution [25].

### Superoxide scavenging activity

Superoxide scavenging activity of the synthesized compounds was determined by the method of Mc Cord & Fridovich [20] (1969), modified by Ruby et al. [21], which depends on the light induced superoxide generation by riboflavin and the corresponding reduction of NBT. The assay mixture contained different concentrations of the test substances and EDTA (6mM containing 3 µg NaCN), NBT (5 µM) and phosphate buffer 58 mM, pH 7.8) in a total vol. of 300 µl. The wells received uniform illumination for 15 min and thereafter optical density was measured at 560 nm [26,27].

In conclusion, a new naturally occurring N<sub>1</sub>, N<sub>6</sub>-dihydrocinnamyl putrescine bisamide, JBIR-94, along with nine structural analogs and a series of substituted phenyl and alkyl putrescine bisamides have been synthesized from putrescine and appropriately substituted carboxylic acids, through carboxylic acid chlorides. Antimicrobial, 5-LOX enzyme inhibitory and antioxidant studies were performed for all synthesized compounds. Dihydrocinnamyl series of putrescine bisamides (4a-4i) showed efficient bioactivities compared to substituted phenyl (6a-6g) and dialkyl (6h-6j) series of compounds. Among the tested compounds, 4a (JBIR-94) and 4h showed potent antimicrobial, and anti-inflammatory activities [28,29].

### Characterization of putrescine bisamides

**N, N'-(Butane-1,4-diyl)bis(3-(4-hydroxy-3-methoxyphenyl) propanamide) (4a).** Gray solid, yield 90%, mp 140-148°C; <sup>1</sup>H NMR (400 MHz, MeOD) δ 6.67 (d, J = 1.6 Hz, 2H), 6.59 (d, J = 8 Hz, 2H), 6.52 (dd, J = 1.6, 8.0 Hz, 2H), 3.72 (s, 6H), 2.97 (brs, 4H), 2.71 (t, J = 7.6 Hz, 4H), 2.32 (t, J = 7.6 Hz, 4H), 1.18 (m, 4H); <sup>13</sup>C NMR (100 MHz, MeOD) δ 175.4, 148.9, 145.9, 133.8, 121.9, 116.3, 113.4, 56.5, 39.9, 39.4, 32.6, 27.6; LC-MS: m/z: 443.3 (M-H<sup>+</sup>); Anal. Calcd. for C<sub>24</sub>H<sub>32</sub>N<sub>2</sub>O<sub>6</sub>; C, 64.85; H, 7.26, N, 6.30 Found: C, 64.80; H, 7.30, N, 6.25.

**N,N'-(Butane-1,4-diyl) bis (3-(3-methoxyphenyl) propanamide) (4b).** White solid, yield 92%, mp 160-166°C; <sup>1</sup>H NMR (400 MHz, MeOD) δ 7.05 (t, J = 8 Hz, 2H), 6.67 (m, 4H), 6.62 (dd, J = 1.2, 8.0 Hz, 2H), 3.65 (s, 6H), 2.98 (t, J = 6.4 Hz, 4H), 2.76 (t, J = 7.6 Hz, 4H), 2.35 (t, J = 7.6 Hz, 4H), 1.22 (brs, 4H); <sup>13</sup>C-NMR (100 MHz, MeOD) δ 175.1, 161.3, 143.7, 130.4, 121.8, 115.2, 112.6, 55.6, 39.9, 38.9, 32.9, 27.6; LC-MS: m/z 411.3 (M-H<sup>+</sup>); Anal. Calcd. for C<sub>24</sub>H<sub>32</sub>N<sub>2</sub>O<sub>4</sub>; C, 69.88; H, 7.82; N, 6.79; Found: C, 69.80; H, 7.886; N, 6.82.

**N,N'-(Butane-1,4-diyl)bis(3-(2-methoxyphenyl)propanamide) (4c).** White solid, yield 92%, mp 150-156°C, <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 7.73 (t, J = 4.8 Hz, 2H), 7.17 (t, J = 7.6 Hz, 2H), 7.12 (d, J = 7.2 Hz, 2H) 6.93 (d, J = 8.0 Hz, 2H), 6.84 (t, J = 7.2 Hz, 2H), 3.78 (s, 6H), 3.01 (d, J = 4.8 Hz, 4H), 2.76 (t, J = 7.6 Hz, 4H), 2.30 (t, J = 8.0 Hz, 4H), 1.33 (brs, 4H); <sup>13</sup>C NMR (100 MHz DMSO-d<sub>6</sub>) δ 171.3, 156.9, 129.3, 129.1, 127.2, 120.2, 110.5, 55.2, 38.1, 35.4, 26.6, 25.7; LC-MS: m/z

413.5 (M+H)<sup>+</sup>, 435.4 (M+Na)<sup>+</sup>; Anal. Calcd. for C<sub>24</sub>H<sub>32</sub>N<sub>2</sub>O<sub>4</sub>, C, 69.88; H, 7.82; N, 6.79; Found: C, 69.78; H, 7.88; N, 6.80.

**N,N'-(Butane-1,4-diyl)bis(3-(4-methoxyphenyl)propanamide) (4d).** White solid, yield 92%, mp 158-162°C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.01 (t, *J* = 5.2 Hz, 2H), 7.49 (d, *J* = 8.4 Hz, 4H), 6.96 (d, *J* = 8.4 Hz, 4H), 3.78 (s, 6H), 2.98 (t, *J* = 6.4 Hz, 4H), 2.75 (t, *J* = 7.6 Hz, 4H), 2.36 (t, *J* = 7.6 Hz, 4H), 1.22 (brs, 4H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 171.2, 156.9, 129.3, 129.1, 127.2, 120.2, 110.5, 53.2, 38.9, 38.1, 35.4, 25.6, 25.7; LC-MS: *m/z* 413.5 (M+H)<sup>+</sup>, 435.4 (M+Na)<sup>+</sup>. Anal. Calcd for. C<sub>24</sub>H<sub>32</sub>N<sub>2</sub>O<sub>4</sub>, C, 69.88; H, 7.82; N, 6.79; Found: C, 69.83; H, 7.87; N, 6.83.

**N,N'-(Butane-1,4-diyl)bis(3-(3,4-dimethoxyphenyl)propanamide (4e).** White solid, yield 90%, mp 176-178°C <sup>1</sup>H NMR (400 MHz, MeOD) δ 6.74 (d, *J* = 8.4 Hz, 2H), 6.71 (s, 2H), 6.64 (d, *J* = 8.0 Hz, 2H), 3.70 (s, 6H), 3.68 (s, 6H), 2.97 (t, *J* = 6.0 Hz, 4H), 2.74 (t, *J* = 7.6 Hz, 4H), 2.34 (t, *J* = 7.6 Hz, 4H), 1.20 (t, brs, 4H); <sup>13</sup>C NMR (100 MHz, MeOD) δ 175.2, 150.5, 149.1, 135.3, 121.8, 113.9, 113.5, 56.7, 56.5, 39.9, 39.2, 32.5, 27.6; LC-MS: *m/z* 495 (M+Na)<sup>+</sup>. Anal. Calcd for. C<sub>26</sub>H<sub>36</sub>N<sub>2</sub>O<sub>6</sub>, C, 66.08; H, 7.68; N, 5.93; Found: C, 66.03; H, 7.73; N, 5.98

**N,N'-(Butane-1,4-diyl)bis(3-(2,3,4-trimethoxyphenyl)propanamide (4f).** White solid, yield 90%, mp 150-154°C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.82 (t, *J* = 5.2 Hz, 2H), 6.89 (d, *J* = 8.4 Hz, 2H), 6.76 (d, *J* = 8.8 Hz, 2H), 3.84 (s, 6H), 3.82 (s, 6H), 3.79 (s, 6H), 3.07 (d, *J* = 5.2 Hz, 4H), 2.77 (t, *J* = 7.6 Hz, 4H), 2.34 (t, *J* = 8 Hz, 4H), 1.39 (brs, 4H); <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 171.2, 151.8, 151.8, 151.2, 141.8, 126.8, 123.4, 107.8, 60.6, 60.2, 55.8, 38.2, 36.3, 26.6, 25.1; LC-MS: *m/z* 555 (M+Na)<sup>+</sup>. Anal. Calcd for. C<sub>28</sub>H<sub>40</sub>N<sub>2</sub>O<sub>8</sub>, C, 63.14; H, 7.57; N, 5.26. Found: C, 63.10; H, 7.62; N, 5.30.

**N,N'-(Butane-1,4-diyl)bis(3-(3,4,5-trimethoxyphenyl)propanamide (4g).** White solid, yield 90%, mp 140-144°C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.76 (t, *J* = 5.2 Hz, 2H), 6.49 (s, 4H), 3.74 (s, 6H), 3.62 (s, 6H) 3.02 (d, *J* = 5.2 Hz, 4H), 2.75 (t, *J* = 7.6 Hz, 4H), 2.35 (t, *J* = 8.0 Hz, 4H), 1.33 (brs, 2H); <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 171.2, 152.7, 137.1, 135.7, 105.5, 59.9, 55.7, 38.1, 37.1, 31.5, 26.6; LC-MS: *m/z* 533.4 (M+H)<sup>+</sup>, 555.4 (M+Na)<sup>+</sup>. Anal. Calcd for. C<sub>28</sub>H<sub>40</sub>N<sub>2</sub>O<sub>8</sub>, C, 63.14; H, 7.57; N, 5.26. Found: C, 63.09; H, 7.64; N, 5.32.

**N,N'-(Butane-1,4-diyl)bis(3-(benzo[*d*][1,3]dioxo)-5-yl)propanamide (4h).** Brown solid, yield 88%, mp 180-184°C; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.75 (t, *J* = 5.6 Hz, 2H), 6.78 (d, *J* = 8.0 Hz, 2H), 6.76 (s, 2H), 6.63 (d, *J* = 7.6 Hz, 2H), 5.94 (s, 4H), 2.99 (m, 4H), 2.72 (t, *J* = 7.6 Hz, 4H), 2.31 (t, *J* = 7.6 Hz, 4H), 1.30 (brs, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 171.1, 147.1, 145.2, 135.2, 120.9, 108.6, 107.9, 100.5, 38.1, 37.3, 30.8, 26.5; LC-MS: *m/z* 463.3 (M+Na)<sup>+</sup>. Anal. Calcd for. C<sub>24</sub>H<sub>28</sub>N<sub>2</sub>O<sub>6</sub>, C, 65.44; H, 6.41; N, 6.36. Found: C, 65.39; H, 6.45; N, 6.40.

**N,N'-(Butane-1,4-diyl)bis(3-(2-chlorophenyl)propanamide (4i).** White solid, yield 86%, mp 162-166°C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.81 (m, 2H), 7.40 (d, *J* = 6.8 Hz, 2H), 7.28-7.14 (m, 6H), 3.01 (brs, 4H), 2.91 (t, *J* = 8.0 Hz, 4H), 2.37 (t, *J* = 7.6 Hz, 4H), 1.31 (brs, 4H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 171.0, 141.4, 130.4, 129.1, 128.2, 127.2, 125.8, 38.2, 37.0, 31.1, 26.6; LC-MS: *m/z* 419.2 (M-H)<sup>-</sup>. Anal. Calcd for. C<sub>22</sub>H<sub>26</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>, C, 62.71; H, 6.22, Cl, 16.83; N, 6.65 Found: C, 62.65; H, 6.28, Cl, 16.88; N, 6.69.

**N,N'-(Butane-1,4-diyl)dibenzamide (6a).** White solid, yield 92%, mp 130-136°C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.49 (t, *J* = 5.2 Hz, 2H), 7.84 (d, *J* = 7.2 Hz, 4H), 7.51 (t, *J* = 7.2 Hz, 2H), 7.44 (t, *J* = 7.2 Hz, 4H), 3.29 (t, *J* = 6.4 Hz, 4H), 1.57 (brs, 4H). <sup>13</sup>C NMR (100 MHz,

DMSO-*d*<sub>6</sub>) δ 166.1, 134.7, 130.9, 128.2, 127.0, 37.9, 26.7; LC-MS: *m/z* 297.4 (M+H)<sup>+</sup>, 329.4 (M+Na)<sup>+</sup>. Anal. Calcd for C<sub>18</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub>: C, 72.95, H, 6.80, N, 9.45. Found C, 72.90, H, 6.85, N, 9.50.

**N,N'-(Butane-1,4-diyl)bis(4-methylbenzamide) (6b).** White solid, yield 92%, mp 200-216°C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.39 (t, *J* = 5.6 Hz, 2H), 7.74 (d, *J* = 8 Hz, 4H), 7.24 (d, *J* = 8 Hz, 4H), 3.27 (q, *J* = 5.6 Hz, 4H), 2.34 (s, 6H), 1.55 (brs, 4H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 165.9, 140.7, 131.9, 128.7, 127.1, 38.1, 26.8, 23.9; LC-MS: *m/z* 325.4 (M+H)<sup>+</sup>, 347.4 (M+Na)<sup>+</sup>. Anal. Calcd for. C<sub>20</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub>: C, 74.04; H, 7.46; N, 8.64. Found: C, 74.00; H, 7.50; N, 8.69.

**N,N'-(Butane-1,4-diyl)bis(3-methoxybenzamide) (6c).** White solid, yield 92%, mp 134-138°C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.39 (brs, 2H), 7.37-7.28 (m, 6H), 7.01 (d, *J* = 7.2 Hz, 2H), 3.73 (s, 6H), 3.25 (t, *J* = 5.2 Hz, 4H), 1.51 (brs, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 165.8, 159.1, 136.1, 129.3, 119.3, 116.8, 112.4, 55.2, 38.9, 26.7; LC-MS: *m/z* 379.4 (M+Na)<sup>+</sup>. Anal. Calcd for. C<sub>20</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>, C, 67.40; H, 6.79; N, 7.86 Found: C, 67.35; H, 6.83; N, 7.90.

**N,N'-(Butane-1,4-diyl)bis(3,5-dimethoxybenzamide) (6d).** White solid, yield 90%, mp 200°C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.47 (t, *J* = 5.6 Hz, 2H) 7.01 (s, *J* = 2.0 Hz, 4H), 6.62 (s, 2H), 3.77 (s, 12H), 3.27 (t, *J* = 5.2 Hz, 4H), 1.55 (brs, 4H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 165.6, 160.3, 136.8, 105.1, 102.9, 55.3, 38.0, 26.6, LC-MS: *m/z* 417 (M+H)<sup>+</sup>. Anal. Calcd for. C<sub>22</sub>H<sub>28</sub>N<sub>2</sub>O<sub>6</sub>: C, 63.45; H, 6.78; N, 6.73. Found C, 63.40; H, 6.82; N, 6.78.

**N,N'-(Butane-1,4-diyl)bis(4-fluorobenzamide) (6e).** White solid, yield 85%, mp 224-230°C <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.56 (t, *J* = 5.6 Hz, 2H), 7.93 (dd, *J* = 5.6, 8.8 Hz, 4H), 7.27 (t, *J* = 8.8 Hz, 4H), 3.28 (t, *J* = 5.2 Hz, 4H), 1.56 (brs, 4H). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 165.0, 161.3, 131.1, 129.7, 115.1, 38.0, 26.6; LC-MS: *m/z* 331 (M-H)<sup>-</sup>. Anal. Calcd for. C<sub>18</sub>H<sub>18</sub>F<sub>2</sub>N<sub>2</sub>O<sub>2</sub>: C, 65.05; H, 5.46; F, 11.43; N, 8.43, Found: C, 65.00; H, 5.51; F, 11.48; N, 8.47.

**N,N'-(Butane-1,4-diyl)bis(2-iodobenzamide) (6f).** White solid, yield 90%, mp 210-218°C <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.36 (t, *J* = 5.2 Hz, 2H), 7.87 (d, *J* = 7.6 Hz, 2H), 7.43 (t, *J* = 7.6 Hz, 2H), 7.31 (dd, *J* = 1.2, 7.6 Hz, 2H), 7.15 (td, *J* = 1.6, 8.0 Hz, 2H), 3.25 (t, *J* = 5.2 Hz, 4H), 1.63 (m, 4H). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 168.7, 143.4, 138.9, 130.5, 127.9, 127.8, 93.4, 38.0, 26.4; LC-MS: *m/z* 549 (M+H)<sup>+</sup>, 571 (M+Na)<sup>+</sup>. Anal. Calcd for. C<sub>18</sub>H<sub>18</sub>I<sub>2</sub>N<sub>2</sub>O<sub>2</sub>: C, 39.44; H, 3.31; I, 46.30; N, 5.11, Found: C, 39.40; H, 3.35; I, 46.34; N, 5.16

**N,N'-(Butane-1,4-diyl)bis(2,4-dichlorobenzamide) (6g).** White solid, yield 90%, mp 232-238°C, <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.49 (t, *J* = 5.6 Hz, 2H), 7.67 (d, *J* = 1.6 Hz, 2H), 7.48 (d, *J* = 2.0, 8.0 Hz, 2H), 7.45 (d, *J* = 8.4 Hz, 2H), 3.25 (4H, t, *J* = 5.2 Hz, 4H), 1.57 (m, 4H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 165.2, 136.1, 134.2, 131.1, 130.1, 129.0, 127.2, 38.0, 26.3; LC-MS: *m/z* 435.5 (M+H)<sup>+</sup>, 457 (M+Na)<sup>+</sup> Anal. Calcd for. C<sub>18</sub>H<sub>16</sub>Cl<sub>4</sub>N<sub>2</sub>O<sub>2</sub>: C, 49.80; H, 3.71; Cl, 32.66; N, 6.45, Found: C, 49.75; H, 3.75; Cl, 38.66; N, 6.49.

**N,N'-(Butane-1,4-diyl)diacetamide (6h).** Pale yellow solid, yield 90%, mp 120-128°C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.82 (s, 2H), 3.00 (s, 4H), 1.77 (s, 6H), 1.35 (s, 4H). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>) 168.8, 38.1, 26.5, 22.4; LC-MS: *m/z* 195 (M+Na)<sup>+</sup> Anal. Calcd for. C<sub>8</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub>, C, 55.79; H, 9.36; N, 16.27, Found: C, 55.74; H, 9.39; N, 16.32.

**N,N'-(Butane-1,4-diyl)dipropionamide (6i).** Pale yellow solid, yield 90%, mp 120°C <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.74 (s, 2H) 3.01 (q, *J* = 5.2 Hz, 4H), 2.05 (q, *J* = 7.2 Hz, 4H), 1.36 (m, 4H), 0.97 (t, *J* = 7.6 Hz, 6H). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 172.7, 38.5, 28.6, 26.6, 9.9. *m/z* 223 (M+Na)<sup>+</sup>. Anal. Calcd for. C<sub>10</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub>, C, 59.97; H, 10.07; N,



13.99, Found: C, 59.92; H, 10.12; N, 14.04.

*N,N'*-(Butane-1,4-diyl)dihexanamide (6j). Pale yellow solid, mp 154-160°C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.77 (brs, 2H), 3.06 (q, *J* = 5.2 Hz, 4H), 2.08 (t, *J* = 7.2 Hz, 4H), 1.53 (m, 4H), 1.41 (brs, 4H), 1.33-1.24 (m, 8H), 0.91 (t, *J* = 7.2 Hz, 6H). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 171.9, 38.1, 35.4, 30.9, 26.7, 24.9, 21.8, 13.8; LC-MS: *m/z* 307 (M+Na)<sup>+</sup>. Anal. Calcd for. C<sub>16</sub>H<sub>32</sub>N<sub>2</sub>O<sub>2</sub>, C, 67.56; H, 11.34; N, 9.85, Found: C, 67.50; H, 11.40; N, 9.89.

## Results and Discussion

In this study, we have synthesized JBIR-94 along with nine structural analogs and a series of substituted phenyl and dialkyl putrescine bisamides for the first time. We have also screened these compounds for antimicrobial, 5-lipoxygenase enzyme inhibitory and antioxidant activities.

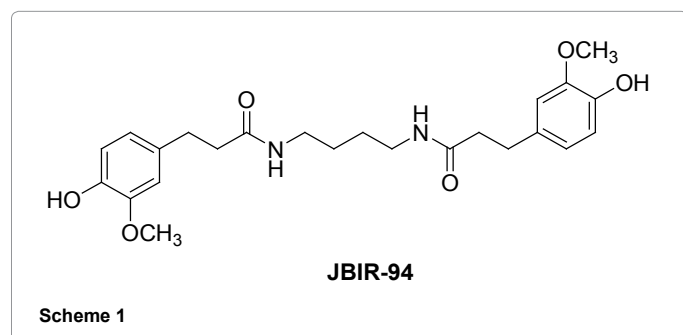
## Chemistry

Benzyl protected ferulic acid was coupled with putrescine via acid chloride which leads to the formation of *N<sub>p</sub>, N<sub>6</sub>*-diferulic putrescine bisamide. Subsequently it was reduced and deprotected with Pd/CaCO<sub>3</sub> to afford the desired product JBIR-94 in good yields (Scheme 1). All the spectral data of the synthesized compound is in good agreement after with isolated compound. The other structural analogues 4b-4i have been synthesized from putrescine and appropriately substituted cinnamic acids as shown in Scheme 1.

Similarly, we have synthesized a series of *N<sub>p</sub>, N<sub>6</sub>*-substituted phenyl and *N<sub>p</sub>, N<sub>6</sub>*-alkyl putrescine bisamides with putrescine by varying aromatic/aliphatic acid chlorides Scheme 2. All the compounds were well characterized by advanced spectroscopic techniques like <sup>1</sup>H NMR, <sup>13</sup>C NMR and Mass (Tables 1 and 2).

## Bioactivity

**Antimicrobial activity:** Antimicrobial studies were carried out on clinical isolates of human pathogenic bacteria (*Salmonella typhi*, *Vibrio cholerae*, *Shigella dysenteriae*, *Enterococcus faecalis* and *Staphylococcus aureus*) and dermatophytic fungi (*Candida albicans*). As shown in Table 3, newly synthesized putrescine bisamides showed significant antibacterial activity but poor anti-fungal activity. Compounds inhibitory zones were found to be in the range between 2-14 mm while 1-1000 µg/ml was the MIC range of compounds. In *N<sub>p</sub>, N<sub>6</sub>*-dihydrocinnamyl putrescine bisamide series (4a-4i), 4h, 4a (JBIR-94) and 4f showed potent antimicrobial activity but compounds, 4c, 4b, 4d, 4e, 4g, and 4i showed moderate activity. Substituted phenyl (6a-6g) and dialkyl (6h-6j) series of putrescine bisamides showed poor antimicrobial activity. Among all the tested compounds, 4h showed highest (14 mm) inhibitory zone and lowest MIC value (1 µg/ml) on *S. typhi*. Moreover it showed comparable potency with Ciprofloxacin



S.No	Entry	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	4Yield(%) <sup>a</sup>
1	4a (JBIR)	H	OMe	OH	H	H	88
2	4b	H	OMe	H	H	H	90
3	4c	OMe	H	H	H	H	90
4	4d	H	H	OMe	H	H	90
5	4e	H	OMe	OMe	H	H	89
6	4f	OMe	OMe	OMe	H	H	88
7	4g	H	OMe	OMe	OMe	H	87
8	4h	H		oCH <sub>2</sub> O	H	H	86
9	4i	Cl	H	H	H	H	85

<sup>a</sup>Isolated yields

**Table 1:** Synthesis of *N<sub>p</sub>, N<sub>6</sub>*-dihydrocinnamyl putrescine bisamides.

S. No	Entry	R	6Yield (%) <sup>a</sup>
1	6a	Ph	92
2	6b	4-Me Ph	92
3	6c	3-OMe Ph	90
4	6d	3,4-diOMe Ph	90
5	6e	4-F Ph	86
6	6f	2-I Ph	88
7	6g	2,4-diCl Ph	86
8	6h	Me	94
9	6i	Et	94
10	6j	Pentyl	92

<sup>a</sup>Isolated yields

**Table 2:** Synthesis of *N<sub>p</sub>, N<sub>6</sub>*-phenyl and *N<sub>p</sub>, N<sub>6</sub>*-alkyl putrescine bisamides.

Entry*	Zone of inhibition (mm)/MIC (µg/ml)					
	<i>S. typhi</i>	<i>V. cholerae</i>	<i>S. dysenteriae</i>	<i>E. faecalis</i>	<i>S. aureus</i>	<i>C. albicans</i>
4a	8/100	8/10	7/100	8/100	9/100	7/100
4b	4/100	5/100	2/100	ND	6/100	6/100
4c	5/1000	4/100	3/100	5/100	4/100	ND
4d	4/100	5/100	4/100	3/1000	4/100	2/100
4e	5/100	6/100	5/100	5/100	6/1000	4/100
4f	7/100	8/1000	7/100	8/100	7/1000	6/1000
4g	5/1000	5/100	6/100	7/1000	8/100	7/100
4h	14/1	13/10	12/10	12/10	13/10	10/100
4i	6/10	7/100	7/1000	7/100	6/100	5/100
6a	5/100	5/100	6/1000	5/1000	7/100	5/100
6b	4/100	5/100	5/100	4/10	5/100	4/100
6c	5/1000	4/1000	6/100	5/1000	4/1000	5/100
6d	5/1000	4/1000	6/100	7/100	5/1000	4/1000
6e	4/1000	5/1000	5/1000	4/1000	5/100	4/1000
6f	3/1000	4/1000	3/100	3/100	4/100	2/1000
6g	2/1000	2/100	3/100	3/1000	4/1000	2/>1000
6h	2/1000	2/1000	4/10	3/100	4/1000	2/1000
6i	3/1000	4/100	3/1000	2/100	4/1000	ND
6j	4/1000	3/1000	5/100	ND	4/1000	ND
Standard*	12/10	12/1	13/10	12/10	11/10	11/10

\*Ciprofloxacin for bacteria and Griseofulvin for dermatophytic fungi; \* 50 µg compound per well; ND: Not Determined

**Table 3:** Antimicrobial activities of newly synthesized putrescine bisamide derivatives against various human pathogens.

which is an antibiotic used as positive control.

**5-Lipoxygenase inhibitory activity:** All synthesized putrescine bisamides were evaluated for 5-Lipoxygenase assay and found to have significant 5-LOX inhibitory activity with IC<sub>50</sub> range from 9.2 to 29.2 µg/ml (Table 4). Dihydrocinnamyl series of compounds (4a-4i), and substituted phenyl (6a-6g) series of compounds showed effective enzyme inhibitory activity with IC<sub>50</sub> range from 9.2 to 14.6 µg/ml,

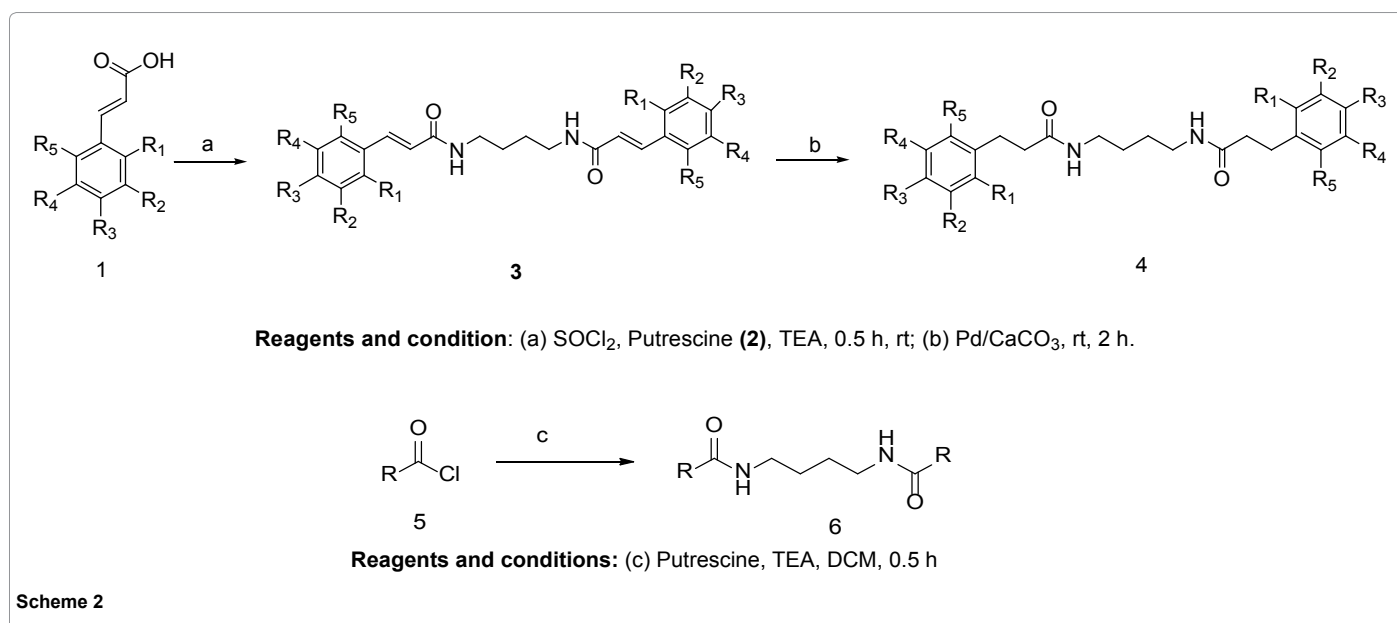
dialkyl series of compounds 6h, 6i and 6j showed dose dependent inhibition with an  $IC_{50}$  of 21, 26.5 and 29.2  $\mu\text{g/ml}$  respectively. These results demonstrated that with the increase in aliphatic carbon chain in dialkyl series causes the decreasing inhibitory activity. Among all the tested compounds 4a (JBIR-94) and 4h (methylenedioxy analog) showed potent inhibitory activity with  $IC_{50}$  of 9.7 and 9.2  $\mu\text{g/ml}$  respectively. Nordihydroguaiaretic acid (positive control) inhibited 5-LOX with  $IC_{50}$  of 4.40  $\mu\text{g/ml}$ .

Further molecular docking studies of all synthesized putrescine bisamides were performed on 5-Lipoxygenase crystal protein (PDB ID 308Y) using iGEM dock programme and found the docking scores (binding energies) range between -98.92 – -141.23 Kcal/mol (Table 4). Among all compounds, 4a and 4h showed good docking efficiency,

which was comparable with Nordihydroguaiaretic acid (Positive control). Docking of 5-LOX with 4a (JBIR-94) showed the binding energy of -138.67 (kcal/mol) and binds the vicinity of amino acid residues present at active site were Leu<sup>188</sup>, Ala<sup>561</sup>, Asn<sup>187</sup> and Ser<sup>562</sup>. 4h binds the vicinity of Leu<sup>188</sup>, Asp<sup>368</sup>, Ser<sup>562</sup>, Asn<sup>187</sup> amino acid residues of 5-LOX crystal protein with the binding energy of -141.23 kcal/mol (Figure 1).

Among all synthesized putrescine bisamides, 4a and 4h showed good *in vitro* 5-LOX inhibition activities and also possess good binding capability with catalytic amino acids of 5-LOX in molecular docking studies (Figure 1).

### Antioxidant activity

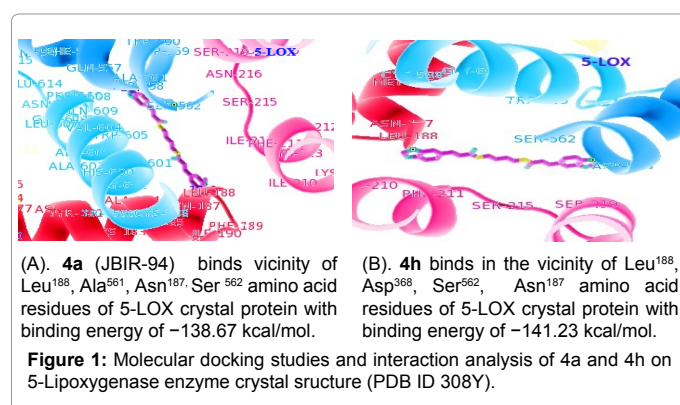


**Scheme 2**

Entry	5-LOX inhibitory activity (Anti-inflammatory activity)		Antioxidant activity (Radical scavenging activity)	
	<i>In vitro</i> studies $IC_{50}$ ( $\mu\text{g/ml}$ )	<i>Insilico</i> studies Dock score (Kcal/mol)	DPPH	Superoxide $IC_{50}$ ( $\mu\text{g/ml}$ )
4a	9.7	-138.67	10.99	9.8
4b	ND	-110.76	8.3	7.8
4c	13.1	-129.32	8.5	7.6
4d	13.3	-128.21	8.8	7.5
4e	14.1	-124.87	10.0	9.1
4f	15.4	-119.67	9.5	9.8
4g	15.3	-118.56	15.4	19.8
4h	9.2	-141.23	5.6	7.6
4i	12.2	-122.65	13.1	14.2
6a	14.6	-126.61	14.2	14.3
6b	13.4	-121.01	10.0	13.1
6c	13.5	-127.21	9.3	10.3
6d	14.4	-129.34	12.2	10.9
6e	10.4	-118.32	15.5	13.03
6f	10.1	-108.49	16.43	15.02
6g	10.1	-119.94	14.45	13.5
6h	21.0	-102.56	8.7	10.3
6i	26.5	-99.23	10.2	11.2
6j	29.2	-98.92	11.2	11.9
Standard*	4.40	-154.92	3.6	3.3

\*Nordihydroguaiaretic acid for 5-LOX, Ascorbic acid for antioxidant activity, ND; Not Determined

**Table 4:** Anti-inflammatory and antioxidant activities of newly synthesized putrescine bisamides.



Antioxidant activities of putrescine bisamides were performed through radical scavenging assay using DPPH and Superoxide radicals. Dihydrocinnamyl (4a-4i), substituted phenyl (6a-6g) and dialkyl (6h-6j) series of putrescine bisamides showed good free radical scavenging activity. Among all the tested compounds, 4h (methylenedioxy analog) was finest radical scavenger with an IC<sub>50</sub> of 5.6 and 7.6 µg/ml for DPPH and Superoxide radicals respectively.

In conclusion, a new naturally occurring N<sub>1</sub>, N<sub>6</sub>-dihydrocinnamyl putrescine bisamide, JBIR-94, along with nine structural analogs and a series of substituted phenyl and alkyl putrescine bisamides have been synthesized from putrescine and appropriately substituted carboxylic acids. Antimicrobial, 5-LOX enzyme inhibitory and antioxidant studies were performed for all synthesized compounds. Dihydrocinnamyl series of putrescine bisamides (4a-4i) showed efficient bioactivities compared to substituted phenyl (6a-6g) and dialkyl (6h-6j) series of compounds. Among the tested compounds, 4h and 4a (JBIR-94) showed good antimicrobial, anti-inflammatory and antioxidant activities.

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