Synthesis, Antibacterial and Lipoxygenase Inhibition Studies of \(N-(\text{Alkyl/aralkyl})-N-(2,3\text{-dihydro-1,4-benzodioxin-6-yl})-4\text{-methylbenzenesulfonamides}\)

\(N-(\text{Alkyl/aralkyl})-N-(2,3\text{-dihydro-1,4-benzodioxin-6-il})-4\text{-metilbenzensülfonamitlerin Sentezi ile Antibakteriyel ve Lipoksijenaz İnhibitör Özellikleri}

Muhammad Athar ABBASI*, Aziz-ur-REHMAN1, Sabahat Z SIDDIQUI1, Anam SHEEA1, Sumaira NAZIR1, Irshad AHMAD2, Rabia MALIK2, Syed AA SHAH3,4

1Government College University, Department of Chemistry, Lahore, Pakistan
2The Islamia University of Bahawalpur, Department of Pharmacy, Bahawalpur, Pakistan
3Universiti Teknologi MARA Faculty of Pharmacy, Puncak Alam Campus, Selangor, Malaysia
4Universiti Teknologi MARA Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Level 9, Selangor, Malaysia

ABSTRACT

Objectives: The present research work was aimed to synthesize some new sulfonamides bearing 1,4-benzodioxin ring, which might have suitable antibacterial potential and can be used as possible therapeutic agents for inflammatory ailments.

Materials and Methods: The synthesis was accomplished by the reaction of 2,3-dihydro-1,4-benzodioxin-6-amine (1) with 4-methylbenzenesulfonyl chloride (2) using 10% aqueous \(\text{Na}_2\text{CO}_3\) to afford \(N-(2,3\text{-dihydro-1,4-benzodioxin-6-yl})-4\text{-methylbenzenesulfonamide}\) (3). Further the parent molecule 3 was reacted with different alkyl/aralkyl halides (4a-e) to achieve \(N-(\text{alkyl/aralkyl})-N-(2,3\text{-dihydro-1,4-benzodioxin-6-yl})-4\text{-methylbenzenesulfonamides}\) (5a-e), using polar aprotic solvent; \(N,N\text{-dimethylformamide}\) (DMF) and catalytic amount of lithium hydride as base. The characterization of synthesized compounds was conducted by contemporary spectral techniques e.g., IR, 1H-NMR and EI-MS. Then these molecules were subjected to screening against various bacterial strains and their inhibitory potential against Lipoxygenase was also ascertained.

Results: The screening results against various Gram-positive and Gram-negative bacterial strains revealed that \(N-(2,3\text{-dihydro-1,4-benzodioxin-6-yl})-4\text{-methylbenzenesulfonamide}\) (3), \(N-(2\text{-bromoethyl})-N-(2,3\text{-dihydro-1,4-benzodioxin-6-yl})-4\text{-methylbenzenesulfonamide}\) (5a) and \(N-(2\text{-phenethyl})-N-(2,3\text{-dihydro-1,4-benzodioxin-6-yl})-4\text{-methylbenzenesulfonamide}\) (5b) showed good inhibitory activity as compared to standard Ciprofloxacin. Moreover, \(N-(3\text{-phenylpropyl})-N-(2,3\text{-dihydro-1,4-benzodioxin-6-yl})-4\text{-methylbenzenesulfonamide}\) (5c) and \(N-(4\text{-chlorobenzyl})-N-(2,3\text{-dihydro-1,4-benzodioxin-6-yl})-4\text{-methylbenzenesulfonamide-amide}\) (5e) displayed decent inhibition against lipoxygenase enzyme relative to standard Baicalein.

Conclusion: On the basis of results obtained it can be concluded that the synthesized sulfonamides may provide an overall indispensable basis to introduce new drug candidates for the cure of inflammatory and other associated diseases.

Key words: 2,3-dihydro-1,4-benzodioxin-6-amine, 1H-NMR, antibacterial potential, lipoxygenase

ÖZ

Amaç: Mevcut araştırma çalışmalarını, uygun antibakteriyel potansiyele sahip olabilen ve inflamatuar hastalıklar için olası terapotik maddeler olarak kullanılabilen, 1,4-benzodoksin halkası taşıyan bazı yeni sülfonamidleri sentezlemek için hazırlanmıştır.

Gereç ve Yöntemler: Sentez, 10% sulu \(\text{Na}_2\text{CO}_3\) kullanılarak 2,3-dihidro-1,4-benzodoksin-6-amin (1) ile 4-metilbenzensülfonil klorit (2) 2-dihidro-1,4-benzodoksin-6-il-4-metilbenzensülfonamit (3). Ayrıca, ana molekül 3, \(N-(\text{alkil/aralkil})-N-(2,3\text{-dihydro-1,4-benzodoksin-6-il})-4\text{-hidroks-4-karboksilik asit elde etmek için farklı alkil/aralkil halojenürler (4a-e) Metilbenzensülfonamlar (5a-e)), polar aprotik çözücü kullanarak; \(N,N\text{-dimethylformamide}\) (DMF) ve baz olarak katalitik miktarda lityum hidrid. Sentezlenen bileşiklerin karakterizasyonu çağdaş spektrum teknikleri (IR, 1H-NMR ve EI-MS) ile gerçekleştirilmiştir. Daha sonra bu moleküller çeşitli bakteri soylarına karşı taramaya tabi tutuldu ve Lipoxygenaz’a karşı önleyici potansiyelleri de tespit edildi.

Bulgular: Çeşitli Gram- pozitif ve Gram- negatif bakteri soylarına karşı tarama sonuçları, \(N-(2,3\text{-dihydro-1,4-benzodoksin-6-il})-4\text{-metilbenzensülfonamit}\) (3), \(N-(\text{Brometil})-N-(2,3\text{-dihydro-1,4-benzodoksin-6-il})-4\text{-metilbenzensülfonamit}\) (5a) ve \(N-(2\text{-fenetil})-N-(2,3\text{-dihydro-1,4-benzodoksin-6-il})-4\text{-metilbenzensülfonamit}\) (5e) 

*Correspondence: E-mail: atrabbs@ yahoo.com, Phone: +92-4211000010-266
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metilbenzensülfonamit (5b) standart Ciprofloxacine kıyasla iyi inhibitör aktivite gösterdi. Ayrıca N-(3-fenilpropil)-N-(2,3-dihidro-1,4-benzodziokin-6-il)-4-metilbenzensülfonamit (5c) ve N-(4-klorobenzil)-N-3-dihidro-1,4-benzodziokin-6-il)-4-metilbenzensülfon-amid (5e), standart Baikaleine göre lipoksjenaz enzimine karşı iyi inhibisyon sergiledi.

Sonuç: Elde edilen sonuçlara dayanarak, sentezlenen sülfanidlerin inflamatuar ve diğer ilişkili hastalıkların tedavisi için yeni ilaç adayları oluşturmak için vazgeçilmez bir temel oluşturabileceğini sonucuna varılırlar.

Anahtar kelimeler: 2,3-dihidro-1,4-benzodiokin-6-amin, 1H-NMR, antibakteriyel potansiyel, lipoksjenaz

INTRODUCTION

Sulfonamides or sulfa drugs bearing SO₂NH- group derived from sulfanilamide, a class of compounds which are being utilized as synthetic antibiotics. In the history of medicines it was amongst the first antibiotic drug which has been used in 1930’s.1 Sulfonamides are also used as antitumor agents, diuretics, anti-inflammatory and exhibits more than two polymorphic forms.2,3,4 Sulfonamides are capable of inhibiting bacterial growth, they also contest against p-aminobenzoic acid for dihydropteroatesynthetase enzyme, which is necessary for the biogenesis of folic acid (required for the growth of cell) by bacteria.5,6 Sulfonamides possess antimicrobial activity against Gram-positive and Gram-negative bacteria and act as carbonic anhydrase inhibitors.7,8,9,10 In combination with Trimethoprim, sulfonamides are used for the treatment of urinary tract infections and prevent parasitic and malarial infections.11 In addition to antiviral agents sulfonamides are also used as antitumor agents, diuretics, anti-inflammatory, antibacterial, and oral hypoglycemic drugs.12,13,14

Sulfasalazine (Figure 1); an antibiotic is used to manage the long-term inflammation of bowel diseases.15 Aliphatic sulfonamide derivatives act as antifungal agents.16 Dioxane rings containing compounds can introduce variety of new substituents into common skeleton and provide new synthetic routes for generation of various organic compounds. These compounds have two special characteristics: (i) under thermal or photochemical conditions they are readily available for alkylethenes (ii) if C-C double bond is present in the dioxane ring then it will act as an enol form of masked acylacetic acids (unit cells in organic synthesis). Some medically important compounds whether synthetic or not, encompass benzodioxane moiety. Compounds encompassing benzodioxane ring system exhibits different biological activities such as, anti-microbial, antioxidant, anti-hepatotoxic and anti-inflammatory.17,18 The incredible pharmacological importance of sulfonamide stimulated us to carry out synthesis and bioactivity studies of N-alkyl/aralkyl-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamides. So, in search of new and potent therapeutic agents, we have synthesized a series of sulfonamides bearing 1,4-benzodioxin ring system. The structures of synthesized compounds were characterized by fourier transform infrared spectroscopy (FTIR), 1H-NMR and EI-MS techniques. Our effort ended fruitful as some of the molecules depicted good inhibitory potential against the some bacterial strains and lipoxigenase enzyme.

EXPERIMENTAL

Measurements

Required chemicals/solvents were of analytical grade and procured from authorized dealers of Sigma Aldrich/Fluka. Thin Layer Chromatography (TLC) coated with silica gel G-25-UV₂₅₄ was used to monitor reactions on every step in various percentages of n-hexane and ethyl acetate as mobile phase. Open capillary tubes were used in Gallen-Kamp melting point apparatus to record the melting points. The spectra of FTIR were recorded on a Jasco-320-A spectrophotometer in KBr disc and the wave number was in cm⁻¹. 1H-NMR spectra were recorded by Bruker spectrometer in CDCl₃ operating at 400 MHz at 25°C. The chemicals shifts (δ) were taken in ppm and coupling constants (J) were recorded in Hertz (Hz). Mass spectra (EI-MS) were measured on Finnigan MAT-312 instrument having the data system.

Synthesis

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (3)

2,3-Dihydro-1,4-benzodioxin-6-amine (1.22 mL; 0.01 mol; 1) and 4-methylbenzenesulfonyl chloride (0.90 g; 0.01 mol; 2) were taken in a round bottom flask having 30 mL of distilled water. The pH of the suspension was adjusted and maintained at 9.0-10.0 by adding aqueous solution of 10% Na₂CO₃ for alkylethenes (ii) if C-C double bond is present in the dioxane ring then it will act as an enol form of masked acylacetic acids (unit cells in organic synthesis). Some medically important compounds whether synthetic or not, encompass benzodioxane moiety. Compounds encompassing benzodioxane ring system exhibits different biological activities such as, anti-microbial, antioxidant, anti-hepatotoxic and anti-inflammatory.17,18 The incredible pharmacological importance of sulfonamide stimulated us to carry out synthesis and bioactivity studies of N-alkyl/aralkyl-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamides. So, in search of new and potent therapeutic agents, we have synthesized a series of sulfonamides bearing 1,4-benzodioxin ring system. The structures of synthesized compounds were characterized by fourier transform infrared spectroscopy (FTIR), 1H-NMR and EI-MS techniques. Our effort ended fruitful as some of the molecules depicted good inhibitory potential against the some bacterial strains and lipoxigenase enzyme.

Figure 1. Structure of Sulfasalazine
dimethyl formamide (DMF) followed by the addition of lithium hydride (LiH) (0.004 g; LiH) in the mixture which was stirred for 2-3 hours at room temperature. After stirring, various alkyl/aralkyl halides (4a-e) were added slowly to the mixture and were further stirred for 2-3 hours. The progress of reaction was monitored via TLC till single spot. After reaction completion the reaction mixture was quenched with cold distilled water to get precipitates of N-(alkyl/aralkyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamides (5a-e) which were collected by the filtration or solvent extraction (using CHCl₃) depending upon the nature of the derived compound.

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (3)

Greyish brown powder; Yield: 82%; m.p: 150°C; Molecular formula: C₁₇H₁₈BrNO₄S; Molecular mass: 430 g/mol; HR-MS: [M]+ 430.9507 (calculated for C₁₇H₁₈BrNO₄S; 430.9508). IR (KBr, cm⁻¹): 3500 (N-H), 2940 (C-H), 1728 (C=O); 1H-NMR (CDCl₃, 400 MHz, δ in ppm): 7.79 (d, J=8.4 Hz, 2H, H-2' & H-3'), 7.21 (t, J=7.6 Hz, 2H, CH₂-7'), 6.96 (d, J=8.4 Hz, 2H, H-5'), 6.86 (d, J=7.6 Hz, 1H, H-8), 2.30 (s, 3H, CH₃-7'); EI-MS (m/z): 423 [M⁺; C₁₇H₁₆BrNO₄S], 381 [C₁₆H₁₄NO₂S]+, 268 [C₁₄H₁₂NO₂S]+, 240 [C₁₃H₁₀NO₂S]+, 197 [C₁₂H₉NO₂S]+, 155 [C₉H₇SO₂]+, 105 [C₈H₅]+, 119 [C₇H₄]+, 91 [C₆H₃]+.

N-(2-Bromoethyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (5a)

Tea pink powder; Yield: 97%; m.p: 142°C; Molecular formula: C₁₇H₁₅NO₂S; Molecular mass: 412 g/mol; HR-MS: [M]+ 412.2992 (calculated for C₁₇H₁₅NO₂S; 412.2996). IR (KBr, cm⁻¹): 3500 (N-H), 2940 (C-H), 1728 (C=O); 1H-NMR (CDCl₃, 400 MHz, δ in ppm): 7.82 (d, J=8.4 Hz, 2H, H-2' & H-3'), 7.16 (d, J=8.0 Hz, 2H, H-8), 2.97 (t, J=7.6 Hz, 2H, CH₂-7'), 2.39 (s, 3H, CH₃-7'); EI-MS (m/z): 412 [M⁺; C₁₇H₁₅NO₂S], 218 [C₁₆H₁₃NO₂S]+, 170 [C₁₅H₁₁O₂]+, 155 [C₁₄H₉SO₂]+, 107 [C₁₂H₇Br]+, 91 [C₆H₃]+.

N-(2-Dibromo-1,4-benzodioxin-6-yl)-N-(2-phenethyl)-4-methylbenzenesulfonamide (5b)

Greyish brown powder; Yield: 85%; m.p: 110°C; Molecular formula: C₂₄H₂₃NO₂S; Molecular mass: 409 g/mol; HR-MS: [M]+ 409.9498 (calculated for C₂₄H₂₃NO₂S; 409.9494). IR (KBr, cm⁻¹): 3500 (N-H), 2940 (C-H), 1728 (C=O); 1H-NMR (CDCl₃, 400 MHz, δ in ppm): 7.57 (d, J=8.4 Hz, 2H, H-2' & H-3'), 7.34 (d, J=8.0 Hz, 2H, H-8), 2.97 (t, J=7.6 Hz, 2H, CH₂-7'), 2.39 (s, 3H, CH₃-7'); EI-MS (m/z): 431 [M⁺; C₂₄H₂₃NO₂S], 218 [C₂₃H₂₁NO₂S]+, 170 [C₂₂H₁₉O₂]+, 155 [C₂₁H₁₇SO₂]+, 107 [C₁₉H₁₅Br]+, 91 [C₆H₃]+.

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-N-(3-phenylpropyl)-4-methylbenzenesulfonamide (5c)

Light brown powder; Yield: 93%; m.p: 130°C; Molecular formula: C₁₉H₂₃NO₂S; Molecular mass: 423 g/mol; HR-MS: [M]+ 423.5260 (calculated for C₁₉H₂₃NO₂S; 423.5267). IR (KBr, cm⁻¹): 3027 (Ar C-H), 1679 (Ar C=C), 1398 (SO₂-), 1681 (C=O).H-NMR (CDCl₃, 400MHz, δ in ppm): 7.57 (d, J=8.4 Hz, 2H, H-2' & H-3'), 7.35 (d, J=8.0 Hz, 2H, H-8), 2.02 (t, J=7.6 Hz, 2H, CH₂-7'), 2.37 (s, 3H, CH₃-7'), 0.93-0.89 (m, 2H, CH₂-8').

N-(2-Chlorobenzyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (5d)

Grey powder; Yield: 96%; m.p: 127°C; Molecular formula: C₂₂H₂₁ClNO₂S; Molecular mass: 429 g/mol; HR-MS: [M]+ 429.9169 (calculated for C₂₂H₂₁ClNO₂S; 429.9176). IR (KBr, cm⁻¹): 3019 (Ar C-H), 1673 (Ar C=C), 1382 (SO₂-), 1095 (C=O), 765 (C-Cl); 1H-NMR (CDCl₃, 400 MHz, δ in ppm): 7.63 (d, J=8.4 Hz, 2H, H-2' & H-6'), 7.36 (dd, J=2.6, 8.2 Hz, 1H, H-3'), 7.30 (d, J=8.0 Hz, 2H, H-2' & H-3'), 5.19 (dd, J=2.6, 8.6 Hz, 1H, H-6'), 7.15-7.10 (m, 2H, H-4'' & H-5''), 6.66 (d, J=8.4 Hz, 1H, H-8), 6.64 (d, J=2.4 Hz, 1H, H-5), 6.55 (dd, J=2.4, 8.8 Hz, 1H, H-7), 4.86 (s, 2H, CH₂-7'), 4.12 (s, 2H, CH₂-2 & CH₂-3).

N-(4-Chlorobenzyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (5e)

Brown powder; Yield: 90%; m.p: 132°C; Molecular formula: C₂₃H₂₃ClNO₂S; Molecular mass: 429 g/mol; HR-MS: [M]+ 429.9171 (calculated for C₂₃H₂₃ClNO₂S; 429.9176). IR (KBr, cm⁻¹):

Antibacterial assay

The antibacterial activity was evaluated by using the referenced method but with minor modifications. The antibacterial activity was carried out in sterile 96-wells microplates under aseptic circumstances. This technique is based on the principle that as the microbial growth increases in a log phase of growth, the number of microbial cells multiply exponentially which in turn increases absorbance of broth medium. Micro organisms used in this study included; three Gram-negative bacteria i.e. Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi.
and two Gram-positive bacteria namely *Bacillus subtilis* and *Staphylococcus aureus*. All the stains were obtained from the local hospital. They are clinically cultured samples/clinical pathogens and were tested and verified by the experts. The tested strains were nourished on stock agar culture medium. The samples being analyzed were diluted in suitable solvents and 20 µL of each sample was pipetted into every well. Fresh bacterial culture maintained overnight was suitably diluted with fresh nutrient broth and was 180 µL quantity of this bacterial culture was poured into every well. The starting absorbance of the culture was strictly maintained at 540 nm between 0.12-0.19. The total volume kept in each well was 200 µL. These microplates covered with lids were incubated for 16-24 hours at 37°C. Before and after incubation, the absorbance was measured at 540 nm using microplate reader, and index of bacterial growth was noted by the difference in absorbance before and after incubation. The formula for calculating the percentage inhibition is:

\[
\text{Inhibition} (\%) = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100
\]

Results are mean of three sets of test samples (n=3, ± standard error of mean). Standard used was ciprofloxacin. Suitable dilutions ranging from 5-30 µg/well were used to measure the minimum inhibitory concentration (MIC). EZ-Fitz Perrella Scientific Inc. Amherst USA software was used to calculate the results.

### Lipoxygenase assay

Lipoxygenase activity was assayed according to the method reported\textsuperscript{24,25,26} with slight modifications. A total volume of 200 µL lipoxygenase assay mixture having 150 µL sodium phosphate buffer (100 mM, pH 8.0), 10 µL test compound and 15 µL purified lipoxygenase enzyme. The contents were mixed and pre read at 234 nm and pre-incubated for 10 min at 25°C. The reaction was initiated by addition of 25 µL substrate solution. The change in absorbance was observed after 6 min at 234 nm. All reactions were performed in triplicates. The positive and negative controls were included in the assay. Baicalein (0.5 mM well\textsuperscript{19}) was used as a positive control.

### Statistical analysis

All the measurements were done in triplicate and statistical analysis was performed by Microsoft Excel 2010. Results are presented as mean ± standard error of mean.

### RESULTS AND DISCUSSION

N-(Alkyl/aralkyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamides (5a-e) were synthesized following pathway sketched in Scheme 1 and Table 1. All conditions suitable for reactions and detailed procedures have been discussed in experimental section. The projected structures of newly synthesized molecules were confirmed via IR, \textsuperscript{1}H-NMR and EI-MS techniques. In search of potent anti-bacterial and lipoxygenase inhibitors, these synthesized molecules were screened against various Gram-positive and Gram-negative bacterial strains (Table 2) and lipoxygenase enzyme (Table 3).

### Table 1. Different alkyl/aralkyl halides (4a-e) utilized in the synthesis of N-(alkyl/aralkyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamides (5a-e)

| Compound | -R | Compound | -R |
|----------|----|----------|----|
| 4a, 5a   | \(-\text{CH}_2-\text{CH}_2-\text{Br}\) | 4c, 5c | \(-\text{H}_2\text{C}=\text{H}_2\text{C}=-\text{H}_2\text{C}\) |
| 4b, 5b   | \(-\text{H}_2\text{C}=-\text{H}_2\text{C}\) | 4d, 5d | \(-\text{H}_2\text{C}=-\text{H}_2\text{C}\) |
| 4e, 5e   | \(-\text{H}_2\text{C}=-\text{H}_2\text{C}\) | 4e, 5e | \(-\text{H}_2\text{C}=-\text{H}_2\text{C}\) |
**Chemistry**

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (3) was reacted with 4-methylbenzenesulfonyl chloride (2) in the presence of 10% Na₂CO₃ under dynamic pH control at 9-10 under stirring for 2-3 hours at room temperature to achieve N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (3). Further, alkyl/aralkylation of the parent compound 3 was done utilizing different alkyl/aralkyl halides (4a-e) in DMF as a polar aprotic solvent and LiH as the base to yield the new target compounds (5a-e). Compound 3 and 5a were synthesized by method reported in literature and the spectral data was also found to be in concordance with the literature data.²⁰ The molecule 5e was obtained as brown powder having melting point 132°C. The molecular formula C₂₂H₂₀ClNO₄S was deduced through its EI-MS, having molecular ion peak at m/z 429 [M⁺] and by counting the number of protons via integration curves in its ¹H-NMR spectrum. The mass spectrum of this molecule has been shown in Figure 2 while its suggested mass fragmentation has been sketched in Figure 3. The IR spectrum showed absorption bands at ν 3039, 1679, 1149, 1379 and 709 cm⁻¹ for the bond stretching of C-H, Ar C-H, Ar C=C, C-O-C, SO₂ and C-Cl respectively. In ¹H-NMR spectrum, two discrete A₂B₂ type spin systems were observed in the aromatic region. The ortho-coupled doublets resonating at δ 7.54 (2H, H-2' & H-6') and δ 7.31 (2H, H-3' & H-5') along with a methyl signal at δ 2.32 (H-7') corroborated the presence of 4-methylbenzenesulfonyl moiety in the molecules. Similarly, the other ortho-coupled doublets at δ 7.13 (2H, H-3'' & H-5'') and δ 7.05 (2H, H-2'' & H-6'') along with a benzylic methylene signal at 4.77 (s, 2H, CH₂-7') were helpful to ascertain the substitution of 4-chlorobenzyl moiety on nitrogen atom of the targeted sulfonamide. The 6-substituted 1,4-benzodioxane nucleus in the molecule was clearly demonstrated by its three typical signals in aromatic region at δ 6.67 (d, J=8.4 Hz, 1H, H-8), 6.61 (d, J=2.4 Hz, 1H, H-5) and 6.52 (d, J=2.4, 8.8 Hz, 1H, H-7) along with a broad singlet in aliphatic region at δ 4.10 (4H, CH₂-2 & CH₂-3). On the basis of above collected evidences, the projected structure of 5e was confirmed as N-(4-chlorobenzyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide. Similarly, the structural analysis of other synthesized molecules (5a-e) was affected in an analogous manner e.g. appearance of an A₂B₂ spin system.

### Table 2. Antibacterial activity (% age inhibition and minimum inhibitory concentration) of synthesized 3 and 5a-e

| Codes | S. typhi | E. coli | B. subtilis |
|-------|---------|--------|------------|
|       | Inhibition % | MIC (µg mL⁻¹) | Inhibition % | MIC (µg mL⁻¹) | Inhibition % | MIC (µg mL⁻¹) |
| 3     | 45.57±0.25 | -      | 79.57±0.53 | 09.22±0.70 | 80.29±0.50 | 08.41±0.98 |
| 5a    | 62.45±0.81 | 13.00±0.89 | 69.14±0.63 | 09.66±0.33 | 70.86±0.64 | 11.46±0.90 |
| 5b    | 57.98±0.56 | 15.72±0.54 | 77.14±0.65 | 10.11±0.04 | 35.14±1.00 | -           |
| 5c    | 48.00±0.24 | -      | 34.86±0.63 | -           | 44.00±0.91 | -           |
| 5d    | 60.38±0.81 | 13.51±0.56 | 46.03±0.68 | -           | 47.81±0.49 | -           |
| 5e    | 56.09±0.48 | 16.12±0.13 | 24.00±0.75 | -           | 34.29±0.54 | -           |
| Ciprofloxacin | 91.05±0.68 | 7.83±0.78 | 92.32±0.42 | 8.01±0.12 | 92.02±0.53 | 7.22±0.67 |

MIC: Minimum inhibitory concentration
for 4-methylbenzenesulfonyl moiety was observed in all the synthesized derivatives. In 5a, the appearance of two triplets at δ 3.83 and 3.36, respectively, marked the amalgamation of bromoethyl moiety at N-atom of the parent sulfonamide. In 5b, the insertion of phenethyl group was confirmed by appearance of a multiplet at δ 7.28-7.12 for phenyl group and two triplets at δ 4.85 and δ 2.02, for two adjacent methylene groups in the molecule. The phenylpropyl group in 5c was characterized by a five-proton multiplet in aromatic region and three methylene signals in aliphatic region. In this case, the central methylene appeared as a multiplet resonating at δ 0.93-0.89. Similarly, the presence of typical signals of 2-chlorobenzyl moiety in 5d, pointed out to the successful thesis of targeted molecule.

Pharmacological screening
Antibacterial activity
Synthesized derivatives were screened for their antibacterial activity against three Gram-negative (Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi) and two Gram-positive bacterial strains (Bacillus subtilis, Staphylococcus aureus). The results of screening are tabulated in Table 2. The synthesized compounds showed moderate antibacterial potential as compared to the standard ciprofloxacin. It was revealed that none of the synthesized compounds showed any activity against Staphylococcus aureus (+) and Pseudomonas aeruginosa (-).

Against S. typhi N-(2-bromoethyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (5a) and N-(2-chlorobenzyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (5d) showed comparatively better inhibition having IC\textsubscript{50} value of 13.00±0.89 µg mL\textsuperscript{-1} and 13.51±0.56 µg mL\textsuperscript{-1} respectively, relative to the reference standard; ciprofloxacin (7.83±0.78 µg mL\textsuperscript{-1}). Parent sulfonamide 3 and N-(2,3-dihydro-1,4-benzodioxin-6-yl)-N-(3-phenylpropyl)-4-methylbenzenesulfonamide (5c) was inactive against S. typhi. Results against E. coli revealed that compounds 3, N-(2-bromoethyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (5a) and N-(2,3-dihydro-1,4-benzodioxin-6-yl)-N-(2-phenethyl)-4-methylbenzenesulfonamide (5b) showed inhibitory potential having IC\textsubscript{50} values of 9.22±0.70 µg mL\textsuperscript{-1} and 9.66±0.33 µg mL\textsuperscript{-1}, respectively, as compared to standard ciprofloxacin (MIC; 8.01±0.12 µg mL\textsuperscript{-1}). Screening results against B. subtilis revealed that Only 3, and N-(2-bromoethyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (5a) showed inhibitory potential with IC\textsubscript{50} values of 8.41±0.98 µg mL\textsuperscript{-1} and 11.6±0.90 µg mL\textsuperscript{-1}, respectively, relative to ciprofloxacin (MIC; 7.22±0.67 µg mL\textsuperscript{-1}). Rest of the compounds did not show any activity against the bacterial strains.

Lipoxygenase activity
All the synthesized compounds were screened against lipoxygenase enzyme. Amongst the screened compounds, N-(2,3-dihydro-1,4-benzodioxin-6-yl)-N-(3-phenylpropyl)-4-methylbenzenesulfonamide (5c) and N-(4-chlorobenzyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (5e) were identified as possible inhibitors of liquid oxygen having IC\textsubscript{50} value of 85.79±0.48 mM and 89.32±0.34 mM respectively, relative to the Baicalein, a reference standard (22.41±1.3 mM). Rest of the compounds showed very low inhibitory potential. The results depicted by the screening are elaborated in Table 3.

CONCLUSION
All the synthesized molecules were achieved in excellent yields by following a simple method. The projected structures of synthesized compounds were well supported by the spectral characterization data by IR, ¹H-NMR and EI-MS. Antibacterial potential of the parent compound 3, and its derivatives 5a-e, revealed that none of the compounds were active against S. aureus and P. aeruginosa. Moreover, N-(2,3-dihydro-1,4-benzodioxin-6-yl)-N-(3-phenylpropyl)-4-methylbenzenesulfonamide (5c) did not show any inhibitory potential against any bacterial strain. Overall, N-(2-bromoethyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (5a) was the only compound which showed maximum inhibition against S. typhi, E. coli and B. subtilis. However, against lipoxygenase enzyme, all compounds showed weaker inhibitory potential except N-(3-phenylpropyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (5c) and N-(4-chlorobenzyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (5e) which displayed decent inhibition against lipoygenase. On the basis of aforesaid results, the synthesized sulfonamides may provide an overall indispensable basis to introduce new drug candidates for the cure of inflammatory and other associated diseases.

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REFERENCES
1. Maren TH. Relations between structure and biological activity of sulfonamides. Ann Rev Pharmacol Toxicol. 1976;16:309-327.

| Table 3. Enzyme inhibition activity (% age inhibition and IC\textsubscript{50}) of synthesized 3 and 5a-e |
| Codes | Lipoygenase assay |
| Code | Conc. (mM) | Inhibition % | IC\textsubscript{50} (mM) |
|---|---|---|---|
| 3 | 0.5 | 86.41±0.58 | 255.38±0.61 |
| 5a | 0.5 | 59.41±0.91 | 405.39±0.39 |
| 5b | 0.5 | 89.93±0.63 | 168.31±0.47 |
| 5c | 0.5 | 98.71±0.42 | 085.79±0.48 |
| 5d | 0.5 | 60.93±0.41 | 314.91±0.25 |
| 5e | 0.5 | 84.71±0.64 | 089.32±0.34 |
| Baicalein | 0.5 | 93.79±1.27 | 22.41±1.30 |
2. Mesley RJ, Houghton EE. Infrared identification of pharmaceutically important sulfonamides with particular reference to the occurrence of polymorphism. J Pharm Pharmac. 1967;19:295-304.

3. Marteneza F, Gomez A. Estimation of the solubility of sulfonamides in aqueous media from partition coefficients and entropies of fusion. Phy and Chem of Liq An Int J. 2010;40:411-420.

4. Delgado DR, Martinez F, Fahkree MAA, Jouyban A. Study of the solubility of some sodium sulfonamides in ethanol + water co-solvent mixtures and correlation with the jouyban-acee model. J Chem Sci. 2011;124:723-730.

5. Reddy NS, Rao S, Chari MA, Kumar VR, Jyothi V, Himabindu V. Synthesis and antibacterial activity of sulfonamide derivatives at C-8 alkyl chain of anacardic acid mixture isolated from a natural product cashew nut shell liquid (CNSL). J Chem Sci. 2011;124:723-730.

6. Winum J, Scozzafqoava A, Montero J, Supuran CT. Therapeutic potential of sulfamides as enzyme inhibitors. Med Res Rev. 2006;26:767-792.

7. Togu V. Acetylcholinesterase: Mechanism of catalysis and inhibition. Curr Med Chem. 2001;1:155-170.

8. Hartman GD, Halczenko W, Prugh JD, Smith RL, Sugrue MF, Mallorga P, Michelson SR, Randall WC, Schwam H, Sondey JM. Thiocoln[2,3-b]furan-2-sulfonamides as topical carbonic anhydrase inhibitors. J Med Chem. 1992;35:3027-3033.

9. Hunt CA, Mallorga PJ, Michelson SR, Schwam H, Sondey JM, Smith RL, Sugrue MF, Shepard KL. 3-substituted thiocoln(2,3-b][1,4]thiazine-6-sulfonamides. A novel class of topically active carbonic anhydrase inhibitors. J Med Chem. 1994;37:240-247.

10. Isik K, Kocak FO. Antimicrobial activity screening of some sulfonamide derivatives on some nocardia species and isolates. Microb Res. 2009;164:49-58.

11. Andrews KT, Fisher GM, Sumanadassa SDM, Skinner-Adams T, Moeker J, Lopeza M, Poulsen S. Antimalarial activity of compounds comprising a primary benzene sulfonamide fragment. Bioorg Med Chem Letters. 2013;23:6114-6117.

12. Mirian M, Zarghi A, Sadeghi S, Tabaraki P, Tavallaee M, Dadrass O, Sadeghi-aliebadali H. Synthesis and cytotoxic evaluation of some novel sulfonamide derivatives against a few human cancer cells. Iran J Pharm. Res. 2011;10:741-748.