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ORIGINAL ARTICLE

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

## *N*-(Alkil/aralkil)-*N*-(2,3-dihidro-1,4-benzodioksin-6-il)-4-metilbenzensülfonamitlerin Sentezi ile Antibakteriyel ve Lipoksijenaz İnhibitör Özellikleri

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## 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 $Na_2CO_3$ to afford *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (**3**). Further the parent molecule 3 was reacted with different alkyl/aralkyl halides (**4a-e**) to achieve *N*-(alkyl/aralkyl)-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamides (**5a-e**), using polar aprotic solvent; *N*,*N*-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-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (**3**), *N*-(2-bromoethyl)-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (**5a**) and *N*-(2-phenethyl)-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (**5b**) showed good inhibitory activity as compared to standard Ciprofloxacin. Moreover, *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-methylbenzenesulfon-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 çalışmaları, uygun antibakteriyel potansiyele sahip olabilen ve inflamatuar hastalıklar için olası terapötik maddeler olarak kullanılabilen, 1,4-benzodioksin halkası taşıyan bazı yeni sülfonamidleri sentezlemek için hazırlanmıştır.

**Gereç ve Yöntemler:** Sentez, 10% sulu $Na_2CO_3$ kullanılarak 2,3-dihidro-1,4-benzodioksin-6-amin (**1**) ile 4-metilbenzensülfonil klorit (**2**) 3-dihidro-l,4-benzodioksin-6-il)-4-metilbenzensülfonamid (**3**). Ayrıca, ana molekül 3, *N*-(alkil/aralkil)-*N*-(2,3-dihidro-l,4-benzodioksin-6-il)-4-hidroksi-4-karboksilik asit elde etmek için farklı alkil/aralkil halojenürler (**4a-e**) Metilbenzensülfonamidler (**5a-e**), polar aprotik çözücü kullanarak; *N*,*N*-dimetilformamit (DMF) ve baz olarak katalitik miktarda lityum hidrid. Sentezlenen bileşiklerin karakterizasyonu çağdaş spektrum teknikleri örneğin IR, 1H-NMR ve EI-MS ile gerçekleştirildi. Daha sonra bu moleküller çeşitli bakteri soylarına karşı taramaya tabi tutuldu ve Lipoksigenaz'a karşı önleyici potansiyelleri de tespit edildi.

**Bulgular:** Çeşitli Gram-pozitif ve Gram-negatif bakteri suşlarına karşı tarama sonuçları, *N*-(2,3-dihidro-1,4-benzodioksin-6-il)-4-metilbenzensülfonamit (**3**), N-Bromoetil)-*N*-(2,3-dihidro-1,4-benzodioksin-6-il)-4-metilbenzensülfonamid (**5a**) ve *N*-(2-fenetil)-*N*-(2,3-dihidro-1,4-benzodioksin-6-il)-4-

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metilbenzensülfonamit (**5b**) standart Ciprofloxacine kıyasla iyi inhibitör aktivite gösterdi. Ayrıca *N*-(3-fenilpropil)-N-(2,3-dihidro-1,4-benzodioksin-6-il)-4-metilbenzensülfonamit (**5c**) ve *N*-(4-klorobenzil)-*N*-3-dihidro-l,4-benzodioksin-6-il)-4-metilbenzensülfon-amid (**5e**), standart Baikaleine göre lipoksijenaz enzimine karşı iyi inhibisyon sergiledi.

**Sonuç:** Elde edilen sonuçlara dayanarak, sentezlenen sülfonamidlerin inflamatuvar ve diğer ilişkili hastalıkların tedavisi için yeni ilaç adayları oluşturmak için vazgeçilmez bir temel oluşturabileceği sonucuna varılabilir.

**Anahtar kelimeler:** 2,3-dihidro-1,4-benzodioksin-6-amin, 1H-NMR, antibakteriyel potansiyel, lipoksijenaz

## INTRODUCTION

Sulfonamides or sulfa drugs bearing $SO_2NH$- 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] Sulfa drugs do not possess any odor and they are mostly white in color or have slightly colored solids, soluble in water 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-leprotic, tuberculostatics 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 alkyletenes (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[17], anti-hepatotoxic and anti-inflammatory.[18,19]

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), $^1$H-NMR and EI-MS techniques. Our effort endured fruitful as some of the molecules depicted good inhibitory potential against the some bacterial strains and lipoxygenase enzyme.

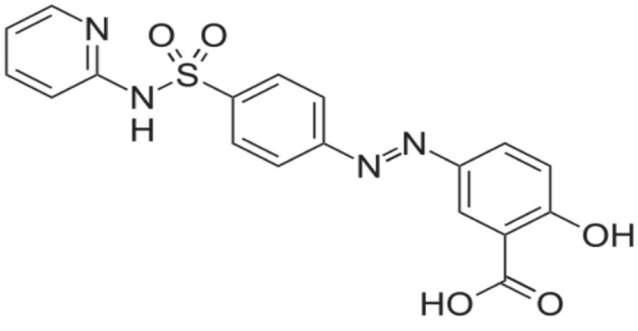



**Figure 1.** Structure of Sulfasalazine

## 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-$UV_{254}$ 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 $cm^{-1}$. $^1$H-NMR spectra were recorded by Bruker spectrometer in $CDCl_3$ 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% $Na_2CO_3$ at room temperature. The reaction solution was stirred for 2-3 hours and progress of the reaction was inspected the by TLC till single spot. The product was obtained by the slow addition of concentrated HCl at pH 2.0-3.0 as brown coloured precipitates which were collected by filtration, washed with distilled water and air-dried to afford pure *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (**3**). The synthesis of compound **3** and its derivative **5a** was coherent with the reported method.[20]

*N-(Alkyl/aralkyl)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamides (**5a-e**)*

*N*-(2,3-Dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (0.1 g; **3**) solubilised in 10 mL of *N*,*N*-

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 $CHCl_3$) 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: $C_{15}H_{15}NO_4S$; Molecular mass: 305 gmol$^{-1}$; HR-MS: $[M]^+$ 305.3509 (calculated for $C_{15}H_{15}NO_4S$; 305.3506). IR (KBr, cm$^1$) $\nu_{max}$: 3500 (N-H), 3080 (Ar C-H), 1650 (Ar C=C), 1390 ($SO_2$-), 1175 (C-O-C); $^1$H-NMR ($CDCl_3$, 400MHz, δ in ppm): 9.87 (s, 1H, NH), 7.58 (d, *J*=8.4 Hz, 2H, H-2' & H-6'), 7.32 (d, *J*=8.0 Hz, 2H, H-3' & H-5'), 6.68 (d, *J*=8.4 Hz, 1H, H-8), 6.65 (d, *J*=2.4 Hz, 1H, H-5), 6.50 (dd, *J*=2.4, 8.8 Hz 1H, H-7), 4.14 (s, 4H, $CH_2$-2 & $CH_2$-3), 2.33 (s, 3H, $CH_3$-7'); EI-MS (m/z): 305 [M$^+$; $C_{15}H_{15}NO_4S$], 241 $[C_{15}H_{15}NO_2]^+$, 218 $[C_{10}H_9NO_2S]^+$, 170 $[C_8H_7O_2]^+$, 155 $[C_7H_7SO_2]^+$, 91 $[C_7H_7]^+$.

*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: $C_{17}H_{18}BrNO_4S$; Molecular mass: 412 gmol$^{-1}$; HR-MS: $[M]^+$ 412.2997 (calculated for $C_{17}H_{18}BrNO_4S$; 412.2992). IR (KBr, cm$^{-1}$) $\nu_{max}$: 3017 (Ar C-H), 1677 (Ar C=C), 1390 ($SO_2$-), 1150 (C-O-C); $^1$H-NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.59 (d, *J*=8.4 Hz, 2H, H-2' & H-6'), 7.37 (d, *J*=8.0 Hz, 2H, H-3' & H-5'), 6.64 (d, *J*=8.4 Hz, 1H, H-8), 6.63 (d, *J*=2.4 Hz, 1H, H-5), 6.53 (dd, *J*=2.4, 8.8 Hz, 1H, H-7), 4.16 (s, 4H, $CH_2$-2 & $CH_2$-3), 3.83 (t, *J*=7.2 Hz, 2H, $CH_2$-1''), 3.36 (t, *J*=7.2 Hz, 2H, $CH_2$-2''), 2.34 (s, 3H, $CH_3$-7'); EI-MS (*m/z*): 412 [M$^+$; $C_{17}H_{18}BrNO_4S$], 321 $[C_{10}H_{11}BrNO_4S]^+$, 318 $[C_{16}H_{16}NO_4S]^+$, 277 $[C_9H_{11}BrNO_2S]^+$, 257 $[C_{10}H_{11}BrNO_2]^+$, 240 $[C_{10}H_9NSO_4]^+$, 218 $[C_{10}H_9NSO_2]^+$, 170 $[C_8H_7O_2]^+$, 155 $[C_7H_7SO_2]^+$, 149 $[C_8H_7NO_2]^+$, 107 $[C_2H_4Br]^+$, 91 $[C_7H_7]^+$.

*N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-N-(2-phenethyl)-4-methylbenzenesulfonamide (**5b**)*

Greyish brown powder; Yield: 85%; m.p: 110˚C; Molecular formula: $C_{23}H_{23}NO_4S$; Molecular mass: 409 gmol$^{-1}$; HR-MS: $[M]^+$ 409.4988 (calculated for $C_{23}H_{23}NO_4S$; 409.4994). IR (KBr, cm$^{-1}$) $\nu_{max}$: 3015 (Ar C-H), 1689 (Ar C=C), 1395 ($SO_2$-), 1155 (C-O-C); $^1$H-NMR ($CDCl_3$, 400MHz, δ in ppm): 7.53 (d, *J*=8.4 Hz, 2H, H-2' & H-6'), 7.34 (d, *J*=8.0 Hz, 2H, H-3' & H-5'), 7.28-7.12 (m, 5H, H-2''' to H-6'''), 6.62 (d, *J*=8.4 Hz, 1H, H-8), 6.60 (d, *J*=2.4 Hz, 1H, H-5), 6.51 (dd, *J*=2.4, 8.8 Hz, 1H, H-7), 4.85 (t, *J*=7.6 Hz, 2H, $CH_2$-8''), 4.15 (s, 4H, $CH_2$-2 & $CH_2$-3), 2.37 (s, 3H, $CH_3$-7'), 2.02 (t, *J*=7.6 Hz, 2H, $CH_2$-7''); EI-MS (*m/z*): 409 $[M]^+$, 345 $[C_{23}H_{23}NO_2]^+$, 332 $[C_{17}H_{18}NO_4S]^+$, 318 $[C_{16}H_{16}NO_4S]^+$, 254 $[C_{16}H_{16}NO_2]^+$, 241 $[C_{15}H_{15}NO_2]^+$, 218 $[C_{10}H_9NSO_4]^+$, 197 $[C_9H_{11}NO_2S]^+$, 155 $[C_7H_7SO_2]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$.

*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: $C_{24}H_{25}NO_4S$; Molecular mass: 423 gmol$^{-1}$; HR-MS: $[M]^+$ 423.5260 (calculated for $C_{24}H_{25}NO_4S$; 423.5267). IR (KBr, cm$^{-1}$) $\nu_{max}$: 3027 (Ar C-H), 1679 (Ar C=C), 1157 (C-O-C), 1398 ($SO_2$-), 681; $^1$H-NMR ($CDCl_3$, 400MHz, δ in ppm): 7.57 (d, *J*=8.4 Hz, 2H, H-2' & H-6'), 7.35 (d, *J*=8.0 Hz, 2H, H-3' & H-5'), 7.27-7.07 (m, 5H, H-2''' to H-6'''), 6.67 (d, *J*=8.4 Hz, 1H, H-8), 6.62 (d, *J*=2.4 Hz, 1H, H-5), 6.57 (dd, *J*=2.4, 8.8 Hz, 1H, H-7), 4.13 (s, 4H, $CH_2$-2 & $CH_2$-3), 2.61 (t, *J*=6.0 Hz, 2H, $CH_2$-9''), 2.30 (s, 3H, $CH_3$-7'), 1.80 (t, *J*=8.0 Hz, 2H, $CH_2$-7''), 0.93-0.89 (m, 2H, $CH_2$-8''); EI-MS (m/z): 423 [M$^+$; $C_{24}H_{25}NO_4S$], 381 $[C_{21}H_{19}NO_4S]^+$, 268 $[C_{17}H_{18}NO_2S]^+$, 240 $[C_{10}H_{10}NO_4S]^+$, 208 $[C_{10}H_{10}NO_2S]^+$, 197 $[C_9H_{11}NO_2S]^+$, 155 $[C_7H_7SO_2]^+$, 105 $[C_8H_9]^+$, 119 $[C_9H_{11}]^+$, 91 $[C_7H_7]^+$.

*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: $C_{22}H_{20}ClNO_4S$; Molecular mass: 429 gmol$^1$; HR-MS: $[M]^+$ 429.9169 (calculated for $C_{22}H_{20}ClNO_4S$; 429.9176). IR (KBr, cm$^1$) $\nu_{max}$: 3019 (Ar C-H), 1673 (Ar C=C), 1382 ($SO_2$-), 1095 (C-O-C), 765 (C-Cl); $^1$H-NMR ($CDCl_3$, 400MHz, δ in ppm): 7.56 (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-3' & H-5'), 7.19 (dd, *J*=2.4, 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, $CH_2$-7''), 4.12 (s, 4H, $CH_2$-2 & $CH_2$-3), 2.35 (s, 3H, $CH_3$-7'); EI-MS (*m/z*): 431 [M+2; $C_{22}H_{20}ClNO_4S]^+$, 429 [M$^+$; $C_{22}H_{20}ClNO_4S$], 365 $[C_{22}H_{20}NO_2Cl]^+$, 274 $[C_{15}H_{13}ClNO_2]^+$, 240 $[C_{10}H_{10}NO_4S]^+$, 208 $[C_{10}H_{10}NO_2S]^+$, 155 $[C_7H_7SO_2]^+$, 125 $[C_7H_6Cl]^+$, 111 $[C_6H_4Cl]^+$, 91 $[C_7H_7]^+$.

*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: $C_{22}H_{20}ClNO_4S$; Molecular mass: 429 gmol$^{-1}$; HR-MS: $[M]^+$ 429.9171 (calculated for $C_{22}H_{20}ClNO_4S$; 429.9176). IR (KBr, cm$^{-1}$) $\nu_{max}$: 3039 (Ar C-H), 1679 (Ar C=C), 1149 (C-O-C), 1379 ($SO_2$-), 769 (C-Cl); $^1$H-NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.54 (d, *J*=8.4 Hz, 2H, H-2' & H-6'), 7.31 (d, *J*=8.0 Hz, 2H, H-3' & H-5'), 7.13 (d, *J*=8.4 Hz, 2H, H-3'' & H-5''), 7.05 (d, *J*=8.4 Hz, 2H, H-2'' & H-6''), 6.67 (d, *J*=8.4 Hz, 1H, H-8), 6.61 (d, *J*=2.4 Hz, 1H, H-5), 6.52 (dd, *J*=2.4, 8.8 Hz, 1H, H-7), 4.77 (s, 2H, $CH_2$-7''), 4.10 (s, 4H, $CH_2$-2 & $CH_2$-3), 2.32 (s, 3H, $CH_3$-7'); EI-MS (m/z): 431 [M+2; $C_{22}H_{20}ClNO_4S]^+$, 429 [M$^+$; $C_{22}H_{20}ClNO_4S$], 365 $[C_{22}H_{20}NO_2Cl]^+$, 274 $[C_{15}H_{13}ClNO_2]^+$, 240 $[C_{10}H_{10}NO_4S]^+$, 208 $[C_{10}H_{10}NO_2S]^+$, 155 $[C_7H_7SO_2]^+$, 125 $[C_7H_6Cl]^+$, 111 $[C_6H_4Cl]^+$, 91 $[C_7H_7]^+$.

*Antibacterial assay*

The antibacterial activity was evaluated by using the referenced method but with minor modifications.[21-23] 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:

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

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[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$^{-1}$) was used as a positive control.

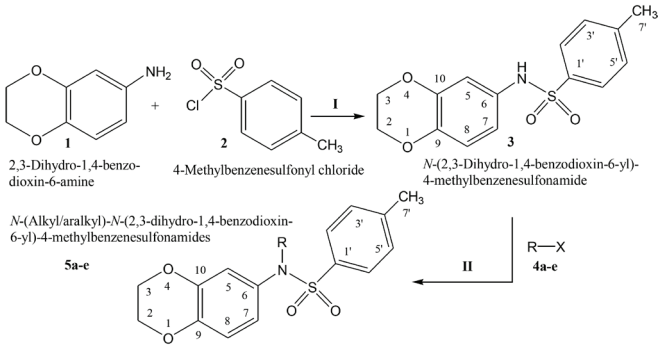
$IC_{50}$ values (concentration at which there is 50% enzyme inhibition) of compounds was calculated using EZ-Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

*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.

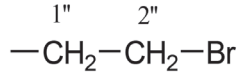
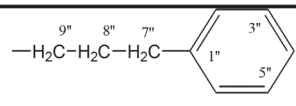
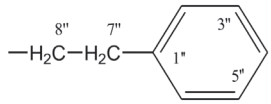
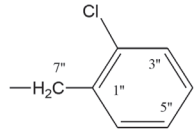
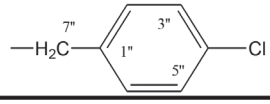
## RESULTS AND DISCUSSION

*N*-(Alkyl/aralkyl)-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfon-amides (**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, $^1$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).




**Scheme 1.** Outline for the synthesis of *N*-(Alkyl/aralkyl)-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamides (5a-e), Reagents & Conditions: (I) Aq. 10% $Na_2CO_3$ soln./pH 9-10/stirring at reverse transcription for 3 hrs, (II) *N, N*-dimethylformamide/LiH/stirring at reverse transcription for 2-3 hrs

**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** |  | **4c, 5c** |  |
| **4b, 5b** |  | **4d, 5d** |  |
| | | **4e, 5e** |  |

*Chemistry*

*N*-2,3-Dihydro-1,4-benzodioxine-6-amine (**1**) was reacted with 4-methylbenzenesulfonyl chloride (**2**) in the presence of 10% $Na_2CO_3$ 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 $C_{22}H_{20}ClNO_4S$ 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 $^1$H-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, $SO_2$ and C-Cl respectively. In $^1$H-NMR spectrum, two discrete $A_2B_2$ 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, $CH_2$-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, $CH_2$-2 & $CH_2$-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 $A_2B_2$ spin system

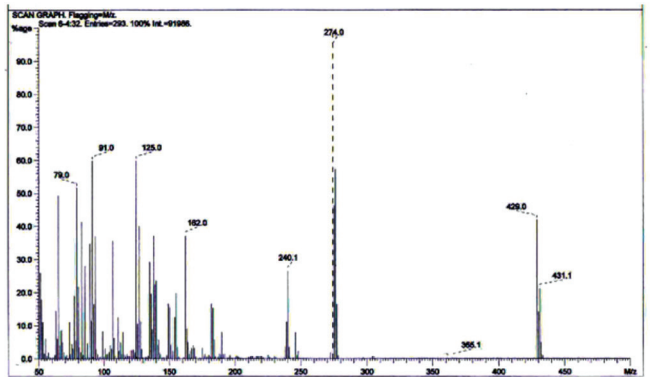


**Figure 2.** EI-MS spectrum of *N*-(4-chlorobenzyl)-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (**5e**)

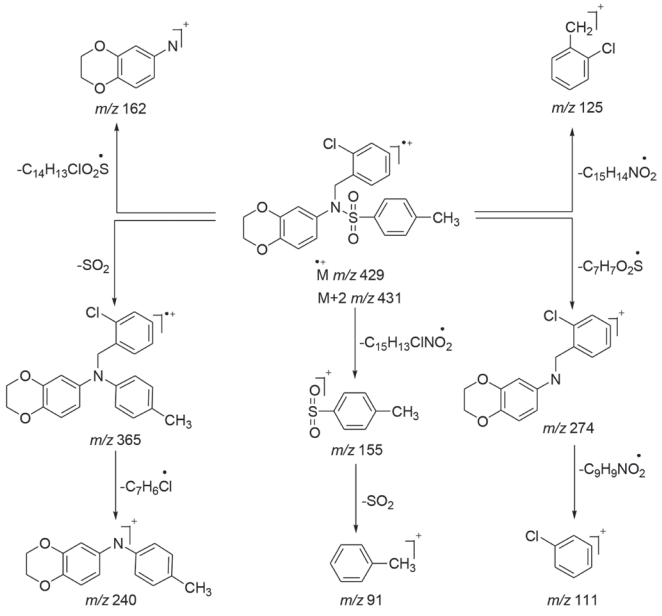



**Figure 3.** Suggested mass fragmentation pattern of *N*-(4-chlorobenzyl)-*N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-4-methylbenzenesulfonamide (**5e**)

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

| Codes | Antibacterial activity | | | | | |
|---|---|---|---|---|---|---|
| | *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±053** | **7.22±0.67** |

MIC: Minimum inhibitory concentration

**Table 3. Enzyme inhibition activity (% age inhibition and $IC_{50}$) of synthesized 3 and 5a-e**

| Codes | Lipoxygenase assay | | |
|---|---|---|---|
| | Conc. (mM) | Inhibition % | $IC_{50}$ (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** |

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 $IC_{50}$ value of 13.00±0.89 μg $mL^{-1}$ and 13.51±0.56 μg $mL^{-1}$ respectively, relative to the reference standard; ciprofloxacin (7.83±0.78 μg $mL^{-1}$). 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 $IC_{50}$ values of 9.22±0.70 μg $mL^{-1}$ and 9.66±0.33 μg $mL^{-1}$, respectively, as compared to standard ciprofloxacin (MIC; 8.01±0.12 μg $mL^{-1}$). 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 $IC_{50}$ values of 8.41±0.98 μg $mL^{-1}$ and 11.46±0.90 μg $mL^{-1}$, respectively, relative to ciprofloxacin (MIC; 7.22±0.67 μg $mL^{-1}$). 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 $IC_{50}$ 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, $^1$H-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 lipoxygenase. 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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*Conflict of Interest: No conflict of interest was declared by the authors.*

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