

Synthesis, Antibacterial and Lipoxygenase Inhibition Studies on Some N-(4-[[Alkyl/aralkyl)(phenethyl)amino]sulfonyl]phenyl)acetamides

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In the present study, 2-phenyl-1-ethanamine (phenethyl amine; **1**) was reacted with 4-acetamidobenzenesulfonyl chloride (**2**) in the presence of 10% aqueous Na₂CO₃ to achieve *N*-{4-[(phenethylamino)sulfonyl]phenyl}acetamide (**3**) which was further reacted with different alkyl/aralkyl halides, **4a-f**, in polar aprotic solvent, *N,N*-dimethylformamide (DMF) and sodium hydride as base to afford various *N*-substituted derivatives of **3**. Structural elucidation of *N*-{4-[(phenethylamino)sulfonyl]phenyl}acetamide derivatives, **5a-f**, was done by IR, ¹H-NMR and mass spectral analysis. Parent molecule **3**, as well as *N*-substituted derivatives **5a**, **5b**, **5e** and **5f** revealed good to moderate antibacterial potential against various **Gram-positive** and **Gram-negative** bacterial strains as compared to standard, **ciprofloxacin**. Moreover, *N*-(4-[[4-chlorobenzyl)(phenethyl)amino]sulfonyl]phenyl)acetamide (**5c**) proved to be a possible inhibitor of lipoxygenase enzyme having IC₅₀ of 135.31±0.81 µM relative to Baicalein which was taken as a reference.

Key words: Phenethyl amine, 4-acetamidobenzenesulfonyl chloride, Antibacterial activity, Lipoxygenase.

Bazı Sentez, Antibakteriyel ve Lipoksijenaz Önleme Çalışmaları *N*-(fenetil)-4-acetamidobenzenesulfonamide *N*-ikame edilmiş türevleri

Mnisbetle IC₅₀ değerine sahip olan, lipoksijenaz enziminin inhibitörü olası olduğu referans. Asetamid (3-Bu çalışmada, fenetil amin (**1**) *N*- elde etmek için (**2**), 10% sulu Na₂CO₃ mevcudiyetinde {[2-fenetil)aminosülfonil] fenil4}4- asetamidobenzenesülfonil klorür ile reaksiyona sokuldu) bundan başka, *N*-ikame edilmiş türevleri elde etmek için temel olarak farklı bir polar aprotik çözücü içinde, bir alkil/ aralkil halojenürler, *N,N*-dimetilformamid (DMF) ve lityum hidrid ile reaksiyona sokuldu . *N*-yapı tayini {4-[(fenetil) sülfonil]fenil} asetamid türevleri, IR, ¹H-NMR ve kütle spektrum analizi ile yapıldı. Ana kısım, *N*-{4-[(fenetilamino)sülfonil]fenil}asetamid (**3**) ve *N*- ikame edilmiş türevleri, **5a**, **5b**, **5e** ve **5f** çeşitli gram-pozitif ve gram-negatif bakteri türlerine karşı antibakteriyel potansiyeli orta iyi ortaya yani standart, siprofloksasin ile karşılaştırıldığında. Ayrıca, *N*-(4-[[4-chlorobenzyl)(fenetil)amino]sülfonil]fenil)asetamid (**5c**) kanıtlanmıştır olarak alınmıştır baicalein için 135.31 ± 0.81 µM.

Anahtar kelimeler: Fenetilamin, 4-acetamidobenzenesulfonyl klorür, Antibakteriyel aktivite, Lipoksijenaz.

INTRODUCTION

Sulfonamide drugs are the pioneer of all the antibacterial drugs in pharmaceutical industry. In start of the 20th century Trypanosomias, a disease common in mice was very progressive which was treated with certain dyes called as azo dyes. These azo dyes were then coined the name of a term “prontosil” by a scientist Bayer AG (1). Later on, Gerhard Domagk studied the effectiveness of prontosil against a streptococcal infection and remained successful in this effort. Sulfonamides are the simple compounds of antibacterial class, composed of general structure R-SO₂-NH₂. Sulfonamides are similar in structure with *para*-amino benzoic acid (PABA) which is an important and essential component for bacterial growth. Bacterial cell cannot absorb folic acid without the formation of a complex PABA-dihydropteroate synthetase. Sulfonamides basically bind and form a complex with dihydropteroate synthetase to inhibit the conversion of folic acid into folate. Sulfonamides might show great antibacterial activity against the strains of **Gram positive** (*Staphylococcus aureus*, and *Bacillus subtilis*) and **Gram negative** bacteria (*Escherichia coli*) (2). Antimicrobial agents are successful in controlling the infectious disease caused by different pathogens (3). Sulfonamides hydroxamic and anilides inhibits the histone deacetylase and acts as anti-tumor agents (3). Benzimidazole derivatives of sulfonamides act as potential anticancer agents to inhibit the growth of malignant tumors (2). Cystein protease is involved in the protein degradation, cell apoptosis. Sulfonamides interfere with the cascade reaction of caspass thus inhibiting the synthesis of protease enzyme (4). Sulfonamides also possess strong activity against HIV proteases. HIV protease is used in combination with transcriptase inhibitors to give Highly Active Antiretroviral Therapy (HART) (4). Carbonic anhydrase are class of enzymes that is responsible for the interconversion of carbon dioxide and bicarbonate. Carbonic anhydrase causes cancer by causing hypoxia thus leading to metastatic spread of cancer cells. Sulfonamides show inhibitory activity against some carbonic anhydrases (5). Cyclooxygenase is involved in the synthesis of prostaglandins. Sulfonamides help in curing the side effects caused by COX-II enzyme. Sulfonamides act as antioxidant (6), and antifungal agents against strains of *Aspergillus niger*, *Fusarium*

oxysporum, and *Candida albicans* (7). Sulfonamides in combination with trimethoprim show great anti-malarial activity (8).

The literature survey has shown that the variation in structure of a molecule affects the biological activity (9-11) which prompted us to synthesize various *N*-substituted sulfonamides using phenethylamine as precursor. Moreover, the synthesized molecules were screened against different bacterial strains and lipoxygenase enzyme to ascertain their therapeutic potential.

EXPERIMENTAL

Measurements

Required chemicals were purchased from Sigma Aldrich. Solvents used in this study were of analytical grades. Thin layer chromatography (TLC) was carried out on pre-coated silica gel G-25-UV₂₅₄ plates by using ethyl acetate and *n*-hexane as mobile phase. Melting points of the synthesized compounds were recorded on a Griffin and George melting point apparatus by open capillary tube and were uncorrected. IR spectra were recorded in KBr pellet method on a Jasco-320-A spectrometer (wave number in cm⁻¹). ¹H-NMR spectra were taken in CDCl₃ through the Bruker spectrometer operating at 400 MHz. Mass spectra (EIMS) was recorded on a JMS-HX-110 spectrometer, with a data system.

Synthesis of N-{4-[(Phenethylamino)sulfonyl]phenyl}acetamide (3)

2-phenyl-1-ethanamine (phenethyl amine; 1.0 mmol; **1**) in 100 mL water was dispersed in a round bottom flask and pH was maintained at 9-10 by (10%) aqueous Na₂CO₃. 4-Acetamidobenzenesulfonyl chloride (1.0 mmol, **2**) was gradually added in reaction mixture which was stirred and monitored with TLC (*n*-hexane: EtOAc; 70:30) till the completion of the reaction. After completion, dilute HCl was added drop wise along with shaking till pH 2.0-3.0. The precipitates appeared were filtered, washed with distilled water and air-dried to afford *N*-{4-[(phenethylamino)sulfonyl]phenyl}acetamide (**3**).

Synthesis of N-(4-[(Alkyl/aralkyl)(phenethyl)amino]sulfonyl)phenyl)acetamides (5a-f)

N-{4-[(Phenethylamino)sulfonyl]phenyl}acetamide (0.2 g; **3**) in 10 mL *N,N*-dimethylformamide was taken in a round bottom flask followed by the addition of NaH (0.004g) as activator base. The reaction mixture was stirred for 0.5 h at room temperature. Different alkyl/aralkyl halides, **4a-f**, were then slowly added and stirred for 2-3 h. The progress of reaction was monitored by TLC. After completion of the reaction, cold distilled water was added to get the precipitates of desired compounds, **5a-f**, or by solvent extraction technique using CHCl₃ as organic phase depending upon the nature of the compound.

N-{4-[(Phenethylamino)sulfonyl]phenyl}acetamide (**3**)

White solid; Yield: 96 %; m.p.107-110 °C; Molecular formula: C₁₆H₁₈N₂O₃S, Molecular weight: 318 g/mol; HR-MS: [M]⁺ 318.3925 (calculated for C₁₆H₁₈N₂O₃S; 318.3926). IR (KBr, ν_{max}, cm⁻¹): 3300 (N-H), 2921 (Ar C-H), 2715 (CH₂ stretching), 2200 (C-N stretching), 1660 (C=O), 1615 (Ar C=C), 1311 (S=O). ¹H-NMR (CDCl₃, 400 MHz, δ in ppm): 7.52 (d, *J* = 8.2 Hz, 2H, H-3' & H-5'), 7.32 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.23-7.10 (m, 5H, H-2 to H-6), 3.24 (t, *J* = 8.0 Hz, 2H, CH₂-8), 2.63 (t, *J* = 8.0 Hz, 2H, CH₂-7), 2.13 (s, 3H, COCH₃). EIMS (*m/z*): 318 [M]⁺, 198 [C₈H₈NO₃S]⁺, 134 [C₈H₈NO]⁺, 119 [C₈H₉N]⁺, 105 [C₈H₉]⁺, 91 [C₇H₇]⁺, 77 [C₆H₅]⁺, 76 [C₆H₄]⁺, 65 [C₅H₅]⁺, 51 [C₄H₃]⁺, 50 [C₄H₂]⁺.

N-(4-[(2-Chlorobenzyl)(phenethyl)amino]sulfonyl)phenyl)acetamide (**5a**)

White solid; Yield: 92 %; m.p. 198-199 °C; Molecular formula: C₂₃H₂₃N₂O₃SCl, Molecular weight: 442 g mol⁻¹; HR-MS: [M]⁺ 442.9593 (calculated for C₂₃H₂₃N₂O₃SCl; 442.9599). IR (KBr, ν_{max}, cm⁻¹): 3250 (N-H), 1665 (C=O), 2852 (Ar C-H), 1490 (Ar C=C), 1260 (S=O), 632 (C-Cl). ¹H-NMR (CDCl₃, 400 MHz, δ in ppm): 7.73 (d, *J* = 8.4 Hz, 2H, H-3' & H-5'), 7.63 (d, *J* = 8.4 Hz, 2H, H-2' & H-6'), 7.41 (dd, *J* = 1.2, 8.0 Hz, 1H, H-3"), 7.29 (br.d, *J* = 7.6 Hz, 1H, H-6"), 7.21-7.18 (m, 5H, H-2 to H-6), 7.16-7.10 (m, 2H, H-4" & H-5"), 4.27 (s, 2H, CH₂-7"), 3.47 (t, *J* = 8.0 Hz, 2H, CH₂-8), 2.65 (t, *J* = 8.0 Hz, 2H, CH₂-7), 2.23 (s, 3H, COCH₃). EI-MS (*m/z*): 446 (C₂₃H₂₃N₂O₃SCl + 4)⁺ [M+4]⁺, 444 (C₂₃H₂₃N₂O₃SCl + 2)⁺ [M+2]⁺, 442 (C₂₃H₂₃N₂O₃SCl)⁺ [M]⁺, 198 [C₈H₈NO₃S]⁺, 134

$[\text{C}_8\text{H}_8\text{NO}]^+$, 125 $[\text{C}_7\text{H}_6\text{Cl}]^+$, 119 $[\text{C}_8\text{H}_9\text{N}]^+$, 105 $[\text{C}_8\text{H}_9]^+$, 99 $[\text{C}_5\text{H}_4\text{Cl}]^+$, 91 $[\text{C}_7\text{H}_7]^+$, 77 $[\text{C}_6\text{H}_5]^+$, 76 $[\text{C}_6\text{H}_4]^+$, 65 $[\text{C}_5\text{H}_5]^+$.

N-(4-[[Benzyl(phenethyl)amino]sulfonyl]phenyl)acetamide (**5b**)

Yellow sticky substance, Yield: 89 %; Molecular formula: $\text{C}_{23}\text{H}_{24}\text{N}_2\text{O}_3\text{S}$, Molecular weight: 408 g mol^{-1} ; HR-MS: $[\text{M}]^+$ 408.5146 (calculated for $\text{C}_{23}\text{H}_{24}\text{N}_2\text{O}_3\text{S}$; 408.5145). IR (KBr, ν_{max} , cm^{-1}): 3249 (N-H), 2250 (C-N), 1649 (C=O), 1488 (C=C), 1261 (S=O). ^1H -NMR (CDCl_3 , 400 MHz, δ in ppm): 7.86 (d, $J = 8.0 \text{ Hz}$, 2H, H-3' & H-5'), 7.80 (br.d, $J = 8.0 \text{ Hz}$, 2H, H-2' & H-6'), 7.26 (m, 3H, H-3'', H-4'' & H-5''), 7.23-7.20 (m, 7H, H-2'', H-6'' & H-2 to H-6), 4.42 (s, 2H, CH_2 -7''), 3.50 (t, $J = 8.0 \text{ Hz}$, 2H, CH_2 -8), 2.65 (t, $J = 8.0 \text{ Hz}$, 2H, CH_2 -7), 2.10 (s, 3H, COCH_3). EI-MS (m/z): 410 ($\text{C}_{23}\text{H}_{24}\text{N}_2\text{O}_3\text{S} + 2$) $^+$ $[\text{M}+2]^+$, 408 ($\text{C}_{23}\text{H}_{24}\text{N}_2\text{O}_3\text{S}$) $^+$ $[\text{M}]^+$, 198 $[\text{C}_8\text{H}_8\text{NO}_3\text{S}]^+$, 134 $[\text{C}_8\text{H}_8\text{NO}]^+$, 119 $[\text{C}_8\text{H}_9\text{N}]^+$, 105 $[\text{C}_8\text{H}_9]^+$, 91 $[\text{C}_7\text{H}_7]^+$, 77 $[\text{C}_6\text{H}_5]^+$, 76 $[\text{C}_6\text{H}_4]^+$, 65 $[\text{C}_5\text{H}_5]^+$, 51 $[\text{C}_4\text{H}_3]^+$, 50 $[\text{C}_4\text{H}_2]^+$.

N-(4-[[4-Chlorobenzyl](phenethyl)amino]sulfonyl]phenyl)acetamide (**5c**)

White sticky substance, Yield: 90 %; Molecular formula: $\text{C}_{23}\text{H}_{23}\text{N}_2\text{O}_3\text{SCl}$, Molecular weight: 442 g mol^{-1} ; HR-MS: $[\text{M}]^+$ 442.9598 (calculated for $\text{C}_{23}\text{H}_{23}\text{N}_2\text{O}_3\text{SCl}$; 442.9599). IR (KBr, ν_{max} cm^{-1}): 3251 (N-H), 2251 (C-N), 1676 (C=O), 1487 (C=C), 1258 (S=O), 633 (C-Cl). ^1H -NMR (CDCl_3 , 400 MHz, δ in ppm): 7.75 (d, $J = 8.4 \text{ Hz}$, 2H, H-3' & H-5'), 7.64 (d, $J = 8.4 \text{ Hz}$, 2H, H-2' & H-6'), 7.26 (d, $J = 8.0 \text{ Hz}$, 2H, H-3'' & H-5''), 7.19-7.13 (m, 5H, H-2 to H-6), 6.94 (d, $J = 6.8 \text{ Hz}$, 2H, H-2'' & H-6''), 4.25 (s, 2H, CH_2 -7''), 3.26 (t, $J = 8.0 \text{ Hz}$, 2H, CH_2 -8), 2.61 (t, $J = 8.0 \text{ Hz}$, 2H, CH_2 -7), 2.12 (s, 3H, COCH_3). EI-MS (m/z): 446 ($\text{C}_{23}\text{H}_{23}\text{N}_2\text{O}_3\text{SCl} + 4$) $^+$ $[\text{M}+4]^+$, 444 ($\text{C}_{23}\text{H}_{23}\text{N}_2\text{O}_3\text{SCl} + 2$) $^+$ $[\text{M}+2]^+$, 442 ($\text{C}_{23}\text{H}_{23}\text{N}_2\text{O}_3\text{SCl}$) $^+$ $[\text{M}]^+$, 198 $[\text{C}_8\text{H}_8\text{NO}_3\text{S}]^+$, 134 $[\text{C}_8\text{H}_8\text{NO}]^+$, 119 $[\text{C}_8\text{H}_9\text{N}]^+$, 105 $[\text{C}_8\text{H}_9]^+$, 91 $[\text{C}_7\text{H}_7]^+$, 77 $[\text{C}_6\text{H}_5]^+$, 76 $[\text{C}_6\text{H}_4]^+$, 65 $[\text{C}_5\text{H}_5]^+$.

N-(4-[[Pentyl(phenethyl)amino]sulfonyl]phenyl)acetamide (**5d**)

Yellow sticky substance, Yield: 86 %; Molecular formula: $\text{C}_{21}\text{H}_{28}\text{N}_2\text{O}_3\text{S}$, Molecular weight: 388 g mol^{-1} ; HR-MS: $[\text{M}]^+$ 388.5255 (calculated for $\text{C}_{21}\text{H}_{28}\text{N}_2\text{O}_3\text{S}$; 388.5256). IR (KBr, ν_{max} cm^{-1}): 3252 (N-H), 2252 (C-N), 1669 (C=O), 1486 (C=C), 1256 (S=O). ^1H -

NMR (CDCl₃, 400 MHz, δ in ppm): 7.83 (d, $J = 8.0$ Hz, 2H, H-3' & H-5'), 7.75 (d, $J = 8.0$ Hz, H-2' & H-6'), 7.78 (s, 1H, NH-COCH₃), 7.24-7.23 (m, 5H, H-2 to H-6), 3.26 (t, $J = 8.0$ Hz, 2H, CH₂-8), 3.14 (t, $J = 7.1$ Hz, 2H, CH₂-1''), 2.65 (t, $J = 8.0$ Hz, 2H, CH₂-7'), 2.26 (quint. $J = 7.1$ Hz, 2H, CH₂-2''), 1.52-1.42 (m, 4H, CH₂-3'' & CH₂-4''), 2.10 (s, 3H, COCH₃), 0.98 (t, $J = 7.1$ Hz, 3H, CH₃-5''). EI-MS (m/z): 390 (C₂₁H₂₈N₂O₃S + 2)⁺ [M+2]⁺, 388 (C₂₁H₂₈N₂O₃S)⁺ [M]⁺, 198 [C₈H₈NO₃S]⁺, 134 [C₈H₈NO]⁺, 119 [C₈H₉N]⁺, 105 [C₈H₉]⁺, 91 [C₇H₇]⁺, 77 [C₆H₅]⁺, 76 [C₆H₄]⁺, 71 [C₅H₄]⁺.

N-(4-[[*(Butan-2-yl)*(phenethyl)amino]sulfonyl]phenyl)acetamide (**5e**)

White sticky substance, Yield: 87 %; Molecular formula: C₂₀H₂₆N₂O₃S, Molecular weight: 374 gmol⁻¹; HR-MS: [M]⁺ 374.4989 (calculated for C₂₀H₂₆N₂O₃S; 374.4988). IR (KBr, ν_{\max} cm⁻¹): 3256 (N-H), 2251 (C-N), 1665 (C=O), 1483 (C=C), 1255 (S=O). ¹H-NMR (CDCl₃, 400 MHz, δ in ppm): 7.79 (d, $J = 8.1$ Hz, 2H, H-3' & H-5'), 7.74 (d, $J = 8.1$ Hz, 2H, H-2' & H-6'), 7.31-7.26 (m, 5H, H-2 to H-6), 4.22 (m, 1H, H-2''), 3.93 (t, $J = 8.0$ Hz, 2H, CH₂-8), 2.86 (d, $J = 8.0$ Hz, 2H, CH₂-7), 2.13 (s, 3H, COCH₃), 1.81-1.77 (m, 2H, CH₂-3''), 1.43 (d, $J = 7.2$ Hz, 3H, CH₃-1''), 0.87 (t, $J = 7.2$ Hz, 3H, CH₃-4''); EI-MS (m/z): 376 (C₂₀H₂₆N₂O₃S + 2)⁺ [M+2]⁺, 374 (C₂₀H₂₆N₂O₃S)⁺ [M]⁺, 198 [C₈H₈NO₃S]⁺, 134 [C₈H₈NO]⁺, 119 [C₈H₉N]⁺, 105 [C₈H₉]⁺, 91 [C₇H₇]⁺, 77 [C₆H₅]⁺, 76 [C₆H₄]⁺, 65 [C₅H₅]⁺, 57 [C₄H₉]⁺.

N-(4-[[*(Propan-2-yl)*(phenethyl)amino]sulfonyl]phenyl)acetamide (**5f**)

Pinkish white sticky substance, Yield: 90 %; Molecular formula: C₁₉H₂₄N₂O₃S, Molecular weight: 360 gmol⁻¹; HR-MS: [M]⁺ 360.4725 (calculated for C₁₉H₂₄N₂O₃S; 360.4725). IR (KBr, ν_{\max} cm⁻¹): 3254 (N-H), 2250 (C-N), 1671 (C=O), 1485 (C=C), 1254 (S=O). ¹H-NMR (CDCl₃, 400 MHz, δ in ppm): 7.83 (d, $J = 8.1$ Hz, 2H, H-3' & H-5'), 7.76 (d, $J = 8.1$ Hz, 2H, H-2' & H-6'), 7.23-7.18 (m, 5H, H-2 to H-6), 3.94 (t, $J = 8.0$ Hz, 2H, CH₂-8), 3.37- 3.31 (m, 1H, H-2''), 2.65 (t, $J = 8.0$ Hz, 2H, CH₂-7), 2.10 (s, 3H, COCH₃), 1.16 (d, $J = 7.2$ Hz, 6H, CH₃-1'' & CH₃-3'') ; EI-MS (m/z) 362 (C₁₉H₂₄N₂O₃S + 2)⁺ [M+2]⁺, 360 (C₁₉H₂₄N₂O₃S)⁺ [M]⁺, 198 [C₈H₈NO₃S]⁺, 134 [C₈H₈NO]⁺, 119 [C₈H₉N]⁺, 105

$[\text{C}_8\text{H}_9]^+$, 91 $[\text{C}_7\text{H}_7]^+$, 77 $[\text{C}_6\text{H}_5]^+$, 76 $[\text{C}_6\text{H}_4]^+$, 65 $[\text{C}_5\text{H}_5]^+$, 51 $[\text{C}_4\text{H}_3]^+$, 50 $[\text{C}_4\text{H}_2]^+$, 43 $[\text{C}_3\text{H}_7]^+$.

Antibacterial Assay

The antibacterial activity was evaluated by using the referenced method but with minor modifications (12-14). 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:

$$\text{Inhibition (\%)} = \frac{X - Y}{X} \times 100$$

Where,

X = Absorbance in control, containing bacterial culture without test sample;

Y = Absorbance of bacterial culture with test sample.

Results are mean of three sets of test samples (n=3, \pm SEM). Standard used was ciprofloxacin. Suitable dilutions ranging from 5-30 μg / well were used to measure the

Minimum inhibitory concentration (MIC). EZ-Fit Perrella Scientific Inc. Amherst USA software was used to calculate the results.

Lipoxygenase Assay

Lipoxygenase activity was assayed according to the methods reported (15-17) 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 minutes at 25 $^{\circ}\text{C}$. 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. Quercetin (0.5 mM well⁻¹) was used as a positive control.

IC₅₀ 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 \pm SEM.

RESULTS AND DISCUSSION

The targeted derivatives of *N*-{4-[(phenethylamino)sulfonyl]phenyl}acetamide (**3**) were synthesized by the protocol given in **Scheme 1, Table 1**. The reaction conditions are discussed in the experimental section. The structures of all the synthesized compounds were confirmed by spectral analysis using IR, ¹H-NMR and EI-MS techniques. The synthesized compounds were screened for their antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Bacillus subtilis* and *Staphylococcus aureus* (**Table 2** and **Table 3**) as well as for anti-enzymatic activity against lipoxygenase enzyme (**Table 4**).

Chemistry

2-Phenyl-1-ethanamine (phenethyl amine; **1**) was reacted with 4-acetamidobenzenesulfonyl chloride (**2**) in the presence of 10% aqueous Na₂CO₃, under dynamic pH control at 9-10 and stirring for 3-4 hours at room temperature to achieve parent molecule *N*-{4-[(phenethylamino)sulfonyl]phenyl}acetamide (**3**) (9). Further coupling of **3** with different alkyl/aralkyl halides, **4a-f**, in DMF solvent and using NaH as a base, yielded targeted derivatives, **5a-f**. The molecule **5a** was obtained as white solid having melting point 198-199 °C. The molecular formula, C₂₃H₂₃N₂O₃SCl, was ascertained through HR-MS showing [M]⁺ peak at 442.9593 (calculated for 442.9599). Molecular formula was also supported when the number of protons was counted *via* integration curves in the ¹H-NMR spectrum of this molecule. The IR spectrum showed absorption bands at 3250, 2852, 1550, 1490, 1260, 632 cm⁻¹ for bond stretching of N-H, ArC-H, C=O, Ar C=C, S=O and C-Cl, respectively. In its EI-MS spectrum, [M+2]⁺ peak was observed at *m/z* 444 while molecular ion, [M]⁺, peak was observed at *m/z* 442. The other distinct fragment peaks at *m/z* 198 (C₈H₈NO₃S)⁺, 134 (C₈H₈NO)⁺, 125 (C₇H₆Cl)⁺, 119 (C₈H₉N)⁺ helped in assigning the structure of the molecule. The mass fragmentation pattern of this molecule is shown in **Fig. 1**. In ¹H-NMR spectrum, two *ortho*-coupled doublets at δ 7.73 (d, *J* = 8.4 Hz, 2H, H-3' & H-5') and δ 7.63 (d, *J* = 8.4 Hz, 2H, H-2' & H-6') with integration of two protons each, confirmed the presence of 1,4-disubstituted benzene ring, with one substituent as acetamido group appearing at δ 2.23 (s, 3H, COCH₃). A multiplet, having integration of five protons, resonated at δ 7.21-7.18 for a phenyl ring and two triplets δ 3.47 (t, *J* = 8.0 Hz, 2H, CH₂-8), and 2.65 (t, *J* = 8.0 Hz, 2H, CH₂-7) corroborated the presence of a phenethyl amine moiety. In addition to this, signals for four aromatic protons at δ 7.41 (dd, *J* = 1.2, 8.0 Hz, 1H, H-3''), 7.29 (br.d, *J* = 7.6 Hz, 1H, H-6''), 7.16-7.10 (m, 2H, H-4'' & H-5'') along with a benzylic methylene signal at δ 4.27 (s, 2H, CH₂-7'') ascertained the presence of 2-chlorobenzyl group in the molecule. So, all the cumulative evidences affirmed the structure of **5a** as *N*-(4-[(2-Chlorobenzyl)(phenethyl)amino]sulfonyl)phenylacetamide. Similarly, the structures of other synthesized molecules were also confirmed by the aforesaid spectral techniques.

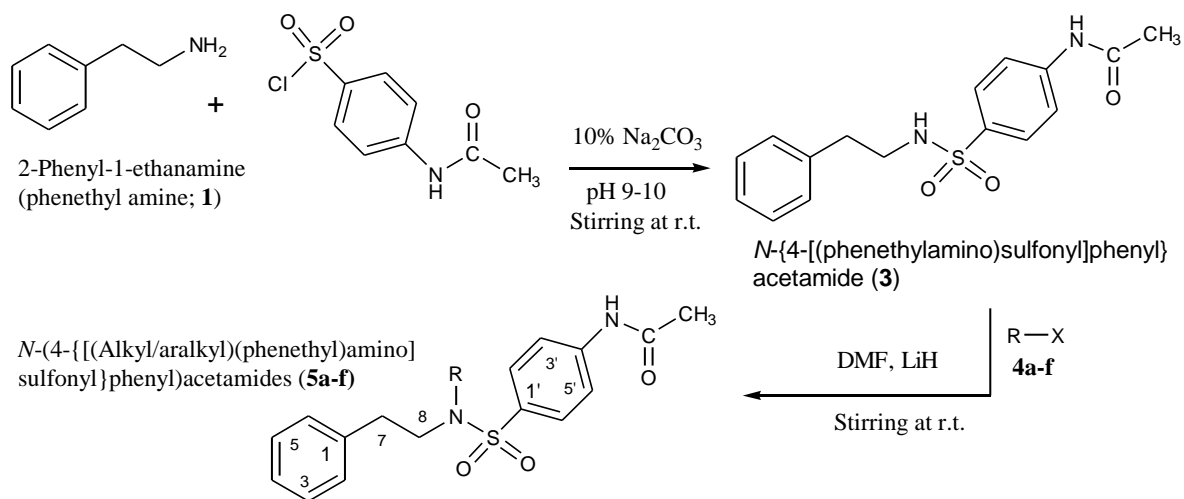


Table-1: Different -R groups in **4a-f** and **5a-f**.

Code	R	Code	R
4a,5a		4d,5d	
4b,5b		4e,5e	
4c,5c		4f,5f	

Scheme-1: Synthesis of targeted *N*-(4-[[Alkyl/aralkyl(phenethyl)amino]sulfonyl]phenyl)acetamides (**5a-f**).

Figure-1: Mass Fragmentation pattern of *N*-(4-[(2-Chlorobenzyl)(phenethyl)amino]sulfonyl)phenyl)acetamide (**5a**).

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 active compounds showed good to moderate antibacterial potential as compared to standard, ciprofloxacin. It was revealed that the parent molecule **3** showed very good inhibitory potential against all the bacterial strains used. Compound **5b** showed good inhibition with MIC value of $10.09 \pm 0.73 \mu\text{g mL}^{-1}$ against *S. typhi* relative to ciprofloxacin standard having MIC value of $7.83 \pm 0.78 \mu\text{g mL}^{-1}$. This good inhibitory potential of **5b** might be attributed to the substitution of benzyl group on nitrogen atom in this molecule. The molecules **5a** and **5e** showed almost similar and reasonably good activity with MIC values of 16.34 ± 0.60 and $10.21 \pm 0.04 \mu\text{g mL}^{-1}$, respectively, against *E. coli*, relative to the standard ciprofloxacin (MIC; $8.01 \pm 0.12 \mu\text{g mL}^{-1}$). Similarly, against *B. subtilis*, parent molecule **3** and **5e** displayed excellent inhibition having MIC values of 9.45 ± 0.88 and $9.38 \pm 0.58 \mu\text{g mL}^{-1}$, respectively, relative to ciprofloxacin (MIC; $7.22 \pm 0.67 \mu\text{g mL}^{-1}$). This activity might probably be due to substitution of butan-2-yl group in **5e** on parent skeleton of molecule **3**. However, the parent molecule, **3**, itself portrayed good activity against *S. aureus*. Compound **5e** also displayed very good activity against *P. aeruginosa* with MIC value of $10.46 \pm 0.95 \mu\text{g mL}^{-1}$. However, compounds **5c** and **5d** remained totally inactive against all the bacterial strains used in the present study. The results are tabulated in **Table 2** and **Table 3**.

Table-2: Antibacterial activity (% age inhibition) of synthesized molecules, **3** and **5a-f**.

Codes	Antibacterial activity (% age inhibition)				
	<i>S. typhi</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
3	66.06±1.25	75.29±0.85	78.00±0.50	75.71±0.87	71.00±1.25
5a	42.25±0.17	51.43±0.58	52.14±1.00	41.17±1.81	73.13±0.17
5b	70.88±0.50	71.00±0.95	74.29±0.57	65.92±0.93	44.50±0.38
5c	49.16±0.72	43.00±1.00	48.90±0.60	45.65±0.69	49.76±1.72
5d	38.14±1.22	49.00±1.13	46.44±0.90	43.28±0.53	38.14±1.22
5e	35.33±0.44	77.71±0.67	78.15±2.00	60.65±1.58	75.38±1.44
5f	60.00±2.13	65.29±0.95	63.02±0.65	61.09±1.00	60.00±2.13
Ciprofloxacin	91.05±0.68	92.32±0.42	92.02±0.53	91.44±0.64	92.50±0.34

Table-3: Antibacterial activity (MIC) of synthesized molecules, **3** and **5a-f**.

Codes	MIC ($\mu\text{g mL}^{-1}$)				
	<i>S. typhi</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
3	11.75±0.99	10.22±0.71	9.45±0.88	10.18±0.88	10.91±0.62
5a	-	16.34±0.60	17.13±0.47	-	11.92±0.55
5b	10.09±0.73	11.10±0.76	11.43±0.90	12.45±0.58	-
5c	-	-	-	-	-
5d	-	-	-	-	-
5e	-	10.21±0.04	9.38±0.58	14.92±0.49	10.46±0.95
5f	13.46 ±0.19	11.66±0.33	13.46 ±0.60	14.64±0.84	13.44±0.86
Ciprofloxacin	7.83±0.78	8.01±0.12	7.22±0.67	7.00±0.54	7.98±0.89

Lipoxygenase Activity

All the synthesized molecules were also screened against lipoxygenase enzyme. The compound *N*-(4-[(4-Chlorobenzyl)(phenethyl)amino]sulfonyl)phenylacetamide (**5c**) was identified as a possible inhibitor of LOX having IC₅₀ value of 135.31±0.81 $\mu\text{g/mL}$ relative to Baicalein, a reference standard, having IC₅₀ value 22.4±1.3 $\mu\text{g/mL}$. Other compounds i.e.

3, **5b**, **5e** and **5f** showed very low activity at the same concentration. The percentage inhibition values ranged from 29.29±0.78 to 38.61±0.92%.

Table-4: Enzyme inhibition activity of synthesized molecules **3** and **5a-f**.

Codes	Lipoxygenase Assay		
	Conc. (mM)	% Inhibition	IC ₅₀ (µg ml ⁻¹)
3	0.5	29.29±0.78	-
5a	0.5	52.41±0.11	471.81±0.68
5b	0.5	-	-
5c	0.5	95.53±0.38	135.31±0.81
5d	0.5	49.73±0.65	> 500
5e	0.5	38.61±0.92	-
5f	0.5	37.86±0.75	-
Baicalein	0.5	93.79±1.27	22.41±1.3

CONCLUSION

The structures of *N*-(4-[[[(Alkyl/aralkyl)(phenethyl)amino]sulfonyl]phenyl)acetamides were well-supported by spectroscopic data. Antibacterial potential of the parent sulfonamide **3** and its derivatives, **5a-f**, revealed that they were exhibiting moderate to good antibacterial potential against all the bacterial strains, except **5c** and **5d** which remained sheer inactive. However, against lipoxygenase enzyme, **5c** was the sole candidate which showed reasonable inhibition, while all remaining compounds rendered weaker inhibition. On the basis of aforesaid results, the synthesized acetamide derivatives provide an overall indispensable basis to introduce new drug candidates for the cure of inflammatory and other associated diseases.

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