Turk J Pharm Sci 12(3), 257-266, 2015

Original article

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-acetamido benzenesulfonyl chloride **(2)** in the presence of 10% aqueous Na_2CO_3 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,^{1}H-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 IC_{50} 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 *N*-(fenetil)-4-acetamidobenzenesulfonamide türevlerinin Sentezi ile Antibakteriyel ve Lipoksijenaz İnhibitör Çalışmaları

Bu çalışmada 2- fenil-1-etanamin **(1)**, N-{[(4-fenetil)aminosülfonil] fenil}-asetamit (**3**) elde etmek üzere 10% sulu Na_2CO_3 mevcudiyetinde 4-asetamidobenzensülfonil klorür (**2**) ile reaksiyona sokulmuş, sonrasında **3** numaralı bileşiğin N-sübstitüe türevlerini elde etmek üzere polar protik solvan DMF ve baz olarak da sodium hidrür kullanılarak farklı alkil halojenürlerle (**4a-f**) muamele edilmiştir. *N*-{4-[(fenetilamino)sülfonil]fenil}asetamid (**5a-f**) türevlerinin yapı aydınlatılması IR,^{1}H-NMR ve mass spektral analizlerle yapılmıştır. Ana bileşik (**3**) ve *N*- sübstitüe türevlerinin (**5a**, **5b**, **5e** ve **5f)** çeşitli gram-pozitif ve gram-negatif bakteri türlerine karşı antibakteriyel potansiyelleri standart, siprofloksasin ile karşılaştırıldığında orta-iyi bulunmuştur. Ayrıca, *N*-(4-{[(4-klorobenzil)(fenetil)amino]sülfonil}fenil)asetamid (**5c**) bileşiğinin baicalein referans alındığında IC_{50}=135.31±0.81 µM değeri ile olası lipoksijenaz inhibitörü olduğu da kanıtlanmıştır.

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

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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-SO_2-NH_2. 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-UV_{254} 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}). ^{1}H-NMR spectra were taken in $CDCl_3$ 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 Na_2CO_3. 4-Acetamido benzenesulfonyl 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 (0.2g; **3**) in 10 mL *N*,*N*-dimethyl formamide 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 $CHCl_3$ 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: $C_{16}H_{18}N_2O_3S$, Molecular weight: 318 g/mol; HR-MS: $[M]^+$ 318.3925 (calculated for $C_{16}H_{18}N_2O_3S$; 318.3926). IR (KBr, ν_{max}, cm^{-1}): 3300 (N-H), 2921 (Ar C-H), 2715 (CH_2 stretching), 2200 (C-N stretching), 1660 (C=O), 1615 (Ar C=C), 1311 (S=O). ^{1}H-NMR ($CDCl_3$, 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, CH_2-8), 2.63 (t, J = 8.0 Hz, 2H, CH_2-7), 2.13 (s, 3H, $CO\underline{CH_3}$). EIMS (*m/z*): 318 $[M]^+$, 198 $[C_8H_8NO_3S]^+$, 134 $[C_8H_8NO]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 76 $[C_6H_4]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$, 50 $[C_4H_2]^+$.

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

White solid; Yield: 92%; m.p. 198-199°C; Molecular formula: $C_{23}H_{23}N_2O_3SCl$, Molecular weight: 442 g/mol; HR-MS: $[M]^+$ 442.9593 (calculated for $C_{23}H_{23}N_2O_3SCl$; 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). ^{1}H-NMR ($CDCl_3$, 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, CH_2-7″), 3.47 (t, J = 8.0 Hz, 2H, CH_2-8), 2.65 (t, J = 8.0 Hz, 2H, CH_2-7), 2.23 (s. 3H, $CO\underline{CH_3}$). EI-MS (*m/z*): 446 $(C_{23}H_{23}N_2O_3SCl + 4)^{\cdot+}$ $[M+4]^+$, 444 $(C_{23}H_{23}N_2O_3SCl + 2)^{\cdot+}$ $[M+2]^+$, 442 $(C_{23}H_{23}N_2O_3SCl)^{\cdot+}$ $[M]^+$, 198 $[C_8H_8NO_3S]^+$, 134 $[C_8H_8NO]^+$, 125 $[C_7H_6Cl]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 99 $[C_5H_4Cl]^+$, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 76 $[C_6H_4]^+$, 65 $[C_5H_5]^+$.

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

Yellow sticky substance, Yield: 89%; Molecular formula: $C_{23}H_{24}N_2O_3S$, Molecular weight: 408 g/mol; HR-MS: $[M]^+$ 408.5146 (calculated for $C_{23}H_{24}N_2O_3S$; 408.5145). IR (KBr, ν_{max}, cm^{-1}): 3249 (N-H), 2250 (C-N), 1649 (C=O), 1488 (C=C), 1261 (S=O). ^{1}H-NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.86 (d, J = 8.0 Hz, 2H, H-3′ & H-5′), 7.80 (br.d, J = 8.0 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 Hz, 2H, CH_2-8), 2.65 (t, J = 8.0 Hz, 2H, CH_2-7), 2.10 (s, 3H, $CO\underline{CH_3}$). EI-MS (*m/z*): 410 $(C_{23}H_{24}N_2O_3S + 2)^{\cdot+}$ $[M+2]^+$, 408 $(C_{23}H_{24}N_2O_3S)^{\cdot+}$ $[M]^+$, 198 $[C_8H_8NO_3S]^+$, 134 $[C_8H_8NO]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 76 $[C_6H_4]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$, 50 $[C_4H_2]^+$.

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

White sticky substance, Yield: 90%; Molecular formula: $C_{23}H_{23}N_2O_3SCl$, Molecular weight: 442 g/mol; HR-MS: $[M]^+$ 442.9598 (calculated for $C_{23}H_{23}N_2O_3SCl$; 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). ^{1}H-NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.75 (d, J = 8.4 Hz, 2H, H-3′ & H-5′), 7.64 (d, J = 8.4

Hz, 2H, H-2′ & H-6′), 7.26 (d, *J* = 8.0 Hz, 2H, H-3″ & H-5″), 7.19-7.13 (*m*, 5H, H-2 to H-6), 6.94 (d, *J* = 6.8 Hz, 2H, H-2″ & H-6″), 4.25 (s, 2H, CH_2-7″), 3.26 (t, *J* = 8.0 Hz, 2H, CH_2-8), 2.61 (t, *J* = 8.0 Hz, 2H, CH_2-7), 2.12 (s, 3H, $CO\underline{CH_3}$). EI-MS (*m/z*): 446 $(C_{23}H_{23}N_2O_3SCl + 4)^{\cdot+}$ $[M+4]^+$, 444 $(C_{23}H_{23}N_2O_3SCl + 2)^{\cdot+}$ $[M+2]^+$, 442 $(C_{23}H_{23}N_2O_3SCl)^{\cdot+}$ $[M]^+$, 198 $[C_8H_8NO_3S]^+$, 134 $[C_8H_8NO]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 76 $[C_6H_4]^+$, 65 $[C_5H_5]^+$.

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

Yellow sticky substance, Yield: 86 %; Molecular formula: $C_{21}H_{28}N_2O_3S$, Molecular weight: 388 g/mol; HR-MS: $[M]^+$ 388.5255 (calculated for $C_{21}H_{28}N_2O_3S$; 388.5256). IR (KBr, ν_{max} cm^{-1}): 3252 (N-H), 2252 (C-N), 1669 (C=O), 1486 (C=C), 1256 (S=O). ^{1}H-NMR ($CDCl_3$, 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, $\underline{NH}$-$COCH_3$), 7.24-7.23 (m, 5H, H-2 to H-6), 3.26 (t, *J* = 8.0 Hz, 2H, CH_2-8), 3.14 (t, *J* = 7.1 Hz, 2H, CH_2-1″), 2.65 (t, *J* = 8.0 Hz, 2H, CH_2-7′), 2.26 (quint. *J* = 7.1 Hz, 2H, CH_2-2″), 1.52-1.42 (m, 4H, CH_2-3″ & CH_2-4″), 2.10 (s, 3H, $CO\underline{CH_3}$), 0.98 (t, *J* = 7.1 Hz, 3H, CH_3-5″). EI-MS *(m/z)*: 390 $(C_{21}H_{28}N_2O_3S + 2)^{\cdot+}$ $[M+2]^+$, 388 $(C_{21}H_{28}N_2O_3S)^{\cdot+}$ $[M]^+$, 198 $[C_8H_8NO_3S]^+$, 134 $[C_8H_8NO]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 76 $[C_6H_4]^+$, 71 $[C_5H_4]^+$.

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

White sticky substance, Yield: 87%; Molecular formula: $C_{20}H_{26}N_2O_3S$, Molecular weight: 374 g/mol; HR-MS: $[M]^+$ 374.4989 (calculated for $C_{20}H_{26}N_2O_3S$; 374.4988). IR (KBr, ν_{max} cm^{-1}): 3256 (N-H), 2251 (C-N), 1665 (C=O), 1483 (C=C), 1255 (S=O). ^{1}H-NMR ($CDCl_3$, 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, CH_2-8), 2.86 (d, *J* = 8.0 Hz, 2H, CH_2-7), 2.13 (s, 3H, $CO\underline{CH_3}$), 1.81-1.77 (m, 2H, CH_2-3"), 1.43 (d, *J* = 7.2 Hz, 3H, CH_3-1"), 0.87 (t, *J* = 7.2 Hz, 3H, CH_3-4"); EI-MS (*m/z*): 376 $(C_{20}H_{26}N_2O_3S + 2)^{\cdot+}$ $[M+2]^+$, 374 $(C_{20}H_{26}N_2O_3S)^{\cdot+}$ $[M]^+$, 198 $[C_8H_8NO_3S]^+$, 134 $[C_8H_8NO]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 76 $[C_6H_4]^+$, 65 $[C_5H_5]^+$, 57 $[C_4H_9]^+$.

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

Pinkish white sticky substance, Yield: 90%; Molecular formula: $C_{19}H_{24}N_2O_3S$, Molecular weight: 360 g/mol; HR-MS: $[M]^+$ 360.4725 (calculated for $C_{19}H_{24}N_2O_3S$; 360.4725). IR (KBr, ν_{max} cm^{-1}): 3254 (N-H), 2250 (C-N), 1671 (C=O), 1485 (C=C), 1254 (S=O). ^{1}H-NMR ($CDCl_3$, 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, CH_2-8), 3.37- 3.31 (m, 1H, H-2″), 2.65 (t, *J* = 8.0 Hz, 2H, CH_2-7), 2.10 (s, 3H, $CO\underline{CH_3}$), 1.16 (d, *J* = 7.2 Hz, 6H, CH_3-1″ & CH_3-3″) ; EI-MS (*m/z*) 362 $(C_{19}H_{24}N_2O_3S + 2)^{\cdot+}$ $[M+2]^+$, 360 $(C_{19}H_{24}N_2O_3S)^{\cdot+}$ $[M]^+$, 198 $[C_8H_8NO_3S]^+$, 134 $[C_8H_8NO]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^+$, 76 $[C_6H_4]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$, 50 $[C_4H_2]^+$, 43 $[C_3H_7]^+$.

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, ± SEM). 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 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°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) 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 ± 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, ^{1}H-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 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 Na_2CO_3, 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 ºC. The molecular formula, $C_{23}H_{23}N_2O_3SCl$, 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 ^{1}H-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 $(C_8H_8NO_3S)^+$, 134 $(C_8H_8NO)^+$, 125 $(C_7H_6Cl)^+$, 119 $(C_8H_9N)^+$ helped in assigning the structure of the molecule. The mass fragmentation pattern of this molecule is shown in Fig. 1. In ^{1}H-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, $CO\underline{CH_3}$). 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, CH_2-8), and 2.65 (t, J = 8.0 Hz, 2H, CH_2-7) 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, CH_2-7′′) 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}phenyl)acetamide*. Similarly, the structures of other synthesized molecules were also confirmed by the aforesaid spectral techniques.

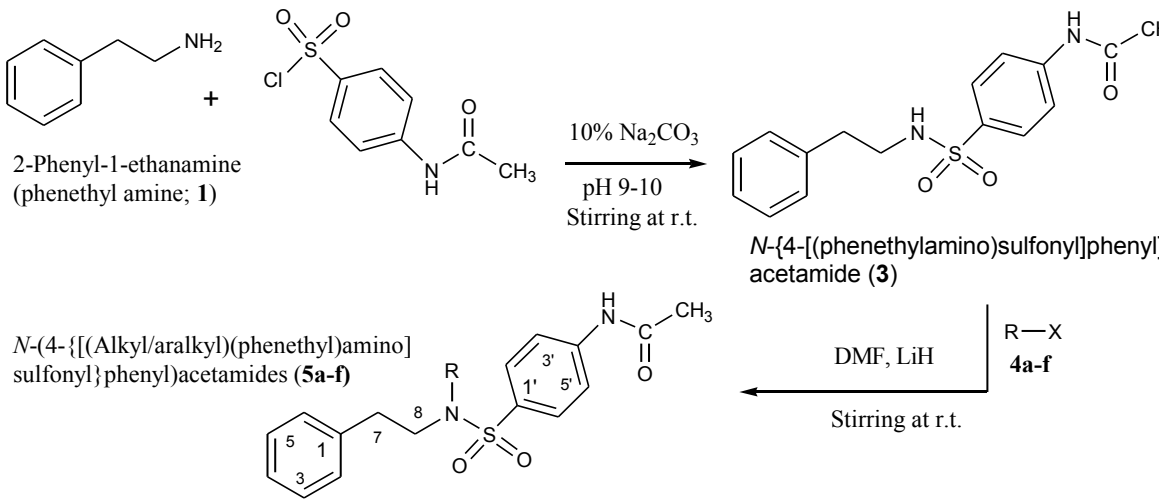
Pharmacological Screening

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±0.73 µg/mL against *S. typhi* relative to ciprofloxacin standard having MIC value of 7.83±0.78 µg/mL. 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±0.04 µg/mL, respectively, against *E. coli,* relative to the standard ciprofloxacin (MIC; 8.01±0.12 µg/mL). Similarly, against *B. subtilis,* parent molecule **3** and **5e** displayed excellent inhibition having MIC values of 9.45±0.88 and 9.38±0.58 µg/mL, respectively, relative to ciprofloxacin (MIC; 7.22±0.67 µg/mL). 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±0.95 µg/mL. 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 3.

Lipoxygenase activity

All the synthesized molecules were also screened against lipoxygenase enzyme. The compound *N-(4-{[(4-Chlorobenzyl)(phenethyl) amino]sulfonyl}phenyl)acetamide* **(5c)** was identified as a possible inhibitor of LOX having IC_{50} value of 135.31±0.81 µg/mL relative to Baicalein, a reference standard, having IC_{50} value 22.4±1.3 µg/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%.



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

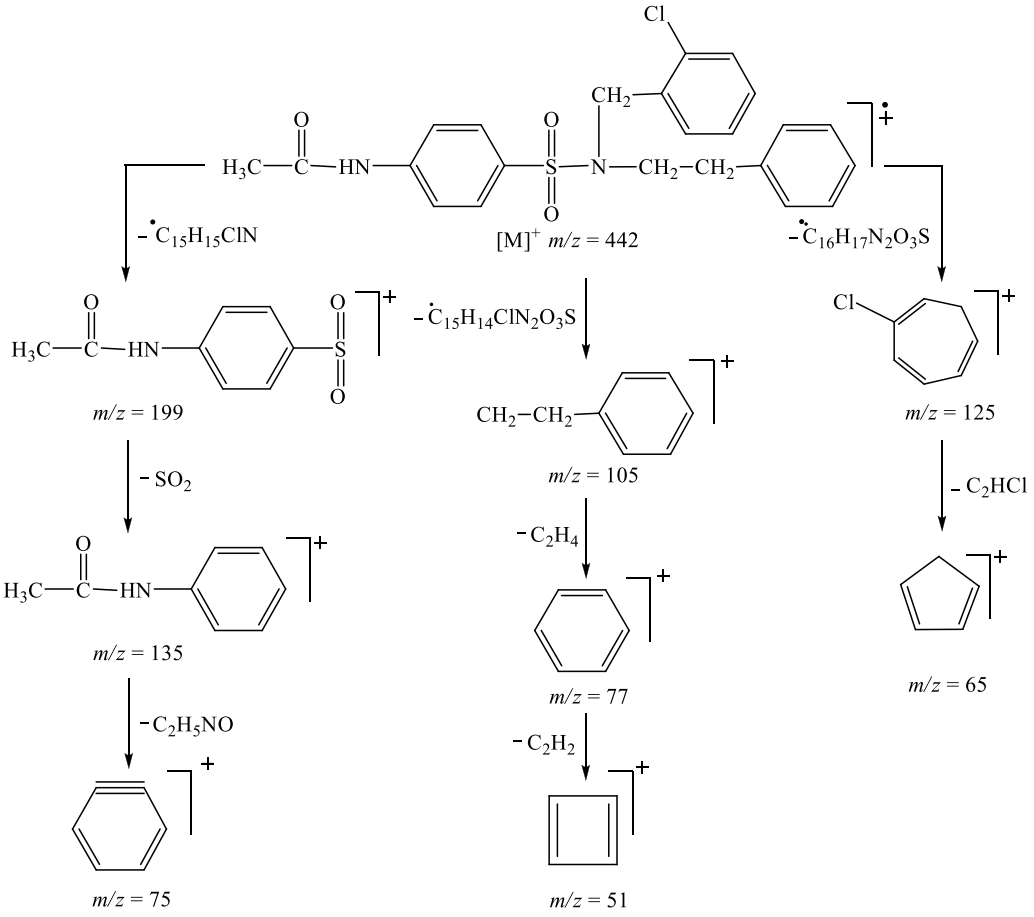


Figure 1. Mass Fragmentation pattern of *N-(4-{[(2-Chlorobenzyl)(phenethyl)amino] sulfonyl}phenyl)acetamide* **(5a)**

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

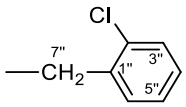
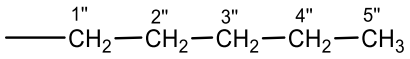
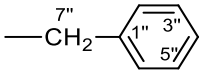
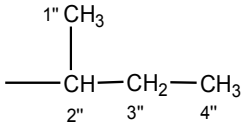
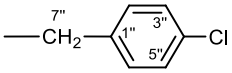
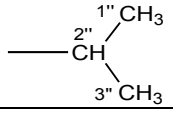
Code	R	Code	R
4a,5a		**4d,5d**	
4b,5b		**4e,5e**	
4c,5c		**4f,5f**	

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

Codes	Antibacterial activity (% age inhibition)				
	S. typhi	***E. coli***	***B. subtilis***	***S. aureus***	***P. aeruginosa***
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±053**	**91.44±0.64**	**92.50±0.34**

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

Codes	MIC (µg mL^{-1})				
	S. typhi	***E. coli***	***B. subtilis***	***S. aureus***	***P. aeruginosa***
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**

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

Codes	Lipoxygenase Assay		
	Conc. (mM)	**% Inhibition**	**IC_{50} ($\mu g\ ml^{-1}$)**
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.

REFERENCES

1. Thomas M Devlin, Textbook of biochemistry, Ed(5), pp. 434,Willey-liss, New York, 2002.
2. Argyropoulou I, Geronikaki A, Vicini P, Zani F, Synthesis and biological evaluation of sulfonamide thiazole and benzothiazole derivatives as antimicrobial agents, ARKIVOC 6 89-102, 2009.
3. Kalidhar U, Kumar R, Kaur A, Synthesis, spectral studies and biological activity of some benzimidazole derivatives containing sulfonamide, Res J Pharm Biol Chem Sci 3, 1072, 2012.
4. Mirian M, Zarghi A, Sadeghi S, Tabaraki T, Tavallaee M, Dadrass O, Sadeghi-aliabadi H, Synthesis and cytotoxic evaluation of some novel sulfonamide derivatives against a few human cancer cells, Int J Pharm Res 10, 741-748, 2011.
5. Cecchi A, Winnum JY, Innocenti A, Vullo D, Montero JL, Sccozaffa A, Supuran CT, Bioorg Med Chem Lett 14, 5775-5780, 2004.
6. Dragostin MO, Lupascu F, Vasile C, Maree M, Nastata V, Morara FR, Picptu D, Profire L, Synthesis and biological evaluation of new 2-azetidinones with sulfonamide structures, Molecules 18, 4140-4157, 2013.
7. Reddy LS, Naresh K, Raju NC, New sulfonamide and carbamate derivatives of 4-(oxiran-2-ylmethoxy)-9H-carbazole: Synthesis, characterization, antimicrobial and antioxidant activities, Der Pharm Lett 5, 221-231, 2013.
8. Andrews KT, Fisher GM, Sumanadassa SDM, Skinner-Adams T, Moeker J, Lopeza M, Poulsena S, Antimalarial activity of compounds comprising a primary beopnzene sulfonamide fragment, Bioorg Med Chem Lett 23, 6114-6117, 2013.
9. Abbasi MA, Ahmad S, Aziz-ur-Rehman, Rasool S, Khan KM., Ashraf M, Nasar R, Ismail T, Sulfonamide derivatives of 2-amino-1-phenylethane as suitable cholinesterase

inhibitors, Trop J Pharm Res 13(5), 739-745, 2014.

10. Abbasi MA, Saeed A, Aziz-ur-Rehman, Khan KM, Ashraf M, Ejaz SA, Synthesis of brominated 2-phenitidine derivatives as valuable inhibitors of cholinesterases for the treatment of Alzheimer's disease, Iran J Pharm Res 13(1), 87-94, 2014.
11. Irshad M, Abbasi MA, Aziz-ur-Rehman, Rasool S, Siddiqui SZ, Ahmad I, Ashraf M, Lodhi MA, Jamal SB, *O*- and *N*-Substituted derivatives of planetol as valuable bioactive compounds, Asian J Chem 26 (4), 1151-1160, 2014.
12. Aziz-ur-Rehman, Nafeesa K, Abbasi MA, Kashfa H, Rasool S, Ahmad I, Arshad S, Synthesis, characterization and biological screening of various *S*-substituted derivatives of 5-(3-nitrophenyl)-1,3,4-oxadiazole-2-thiol, Pak J Chem 3, 1-8, 2013.
13. Kaspady M, Narayanaswamy VK, Raju M, Rao GK, Synthesis, antibacterial Aativity of 2,4-disubstituted oxazoles and thiazoles as bioesters, Lett Drug Des Discov 6, 21-28, 2009.
14. Yang CR, Zang Y, Jacob MR, Khan SI, Zhang YJ, Li XC, Antifungal activity of C-27 steroidal saponins, Antimicrob Agents Ch 50, 1710-1714, 2006.
15. Tappel AL, The mechanism of oxidation of unsaturated fatty acids catalyzed by hematin compounds, Arch Biochem Biophys 44(2), 378-395, 1953.
16. Evans AT, Action of cannabis constituents on enzymes of arachidonate, mechanism: anti-inflammatory potential, Biochem Pharm 36, 2035-2037, 1987.
17. Baylac S, Racine P, Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts, Int J Aromather 13, 138-142, 2003.

Received: 06.11.2014
Accepted: 26.03.2015