World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086

Available online at: http://www.wjpsonline.com/
Research Article

In vitro enzyme inhibition studies on new sulfonamide derivatives of 4-tosyl chloride

Muhammad Athar Abbasi[1,*], Naeem Raza[1], Aziz-ur-Rehman[1], Shahid Rasool[1], Khalid Mohammed Khan[2], Muhammad Ashraf[3], Umber Alam[3], Rumana Nasar[3]

[1]Department of Chemistry, Government College University, Lahore-54000, Pakistan
[2]HEJ Research Institute of Chemistry, ICCBS, University of Karachi, Karachi-75270, Pakistan
[3]Department of Biochemistry & Biotechnology, The Islamia University of Bahawalpur, 63100, Pakistan

Received: 12-11-2013 / Revised: 23-11-2013 / Accepted: 12-01-2014

ABSTRACT

Sulfonamides are considered to be pharmaceutically important class of compounds. In the present work, *N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (**3**) was synthesized by the reaction of 2,4-dimethylaniline (**1**) and 4-tosyl chloride (**2**; 4-methylbenzenesulfonyl chloride) using 10% aqueous Na_2CO_3 solution as reaction medium. At the second step, the synthesized molecule **3** was made to react with different alkyl/aralkyl halides (**4a-o**) to yield the target compounds, **5a-o**, using *N*,*N*-dimethylformamide (DMF) as reaction medium and lithium hydride as an activator. The synthesis of all the compounds was verified by spectral techniques using IR, ^{1}H-NMR and EIMS; and further examined for their anti-enzymatic activities. The synthesized compound **5f** represented a suitable inhibitory potential against α-glucosidase and lipoxygenase enzymes.

Key Words: 2,4-Dimethylaniline, 4-Tosyl Chloride, α-Glucosidase enzyme, Lipoxygenase enzyme

INTRODUCTION

Sulfonamides are an important category of pharmaceutical compounds with a broad spectrum of biological activities [1]. These molecules are broadly used in the pharmaceutical industry as antibacterial agent because they are cheap and cheerful. These are also commonly co-administered to animals or used as feed additives to promote growth in livestock [2]. Sulfonamide drugs have broad applications in the field of medicine, as good antibacterials, diuretics, anticonvulsants, hypoglycemics, and HIV protease inhibitors [3]. Sulfonamide antibiotics are among the most common instigators of allergic or hypersensitivity reactions. Sulfonamides are widely employed to treat microbial infections by inhibiting the growth of gram negative and gram positive bacteria, some protozoa and fungi. Overdose or mingle action of sulfonamide with supplementary drug lead brutal occurrence or even fatal intoxication [4]. More recently, sulfonamides have been found to be potent cysteine protease inhibitors, which could possibly extend their therapeutic applications to include conditions such as Alzheimer's disease, arthritis and cancer [5]. However, sulfonamides have some disadvantages, as they are not handled easily and not suitable for long-term storage [6]. Novel sulfonamide derivatives having CNS (Central Nervous System Disease) activity, procedures for their preparation and use as medicaments are disclosed [7].

α-Glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20) belongs to a family of hydrolase enzymes, found in the brush-border surface membrane of small intestinal cells [8]. This enzyme hydrolyzes the 1,4-glycosidic linkage from the non-reducing end of the α-glucosides, α-linked oligosaccharide and α-glucans substrates to yield α-D-glucose along with other monosaccharides, which are the source of carbon and energy [9]. α-Glucosidase inhibitors are generally utilized for patients with type-2 diabetic mellitus as oral anti-diabetic drugs [10]. These can retard the excretion of D-glucose of oligosaccharides and disaccharides from dietary complex carbohydrates and so detain glucose absorption which results in reduced postprandial

***Corresponding Author Address:** Dr. Muhammad Athar Abbasi, Department of Chemistry, Government College University, Lahore-54000, Pakistan; E-mail: atrabbasi@yahoo.com; abbasi@gcu.edu.pk*

hyperglycemia [11]. Postprandial hyperglycemia has an important role in the development of type-2 diabetes [10]. Hence the inhibition of α-glucosidase enzyme is an important step in managing type-2 diabetes.

In lipoxygenase type-1 (LOX, EC 1.13.11.12), the iron is oxidized from divalent state to the catalytically active trivalent state by the reaction product 15-hydroperoxy-eicosatetraenoic acid (15-HPETE) and leukotrienes from arachidonic acid as a substrate, and 13- hydroperoxy-octadecadienoic acid (13-HPODE) from linoleic acid as a substrate [12]. Leukotrienes are important biologically active mediators in a variety of inflammatory events. It has been found that these LOX products play a key role in variety of disorders such as bronchial asthma, inflammation [13].

The work being proceeded on such type of molecules by our group [14,15] to introduce new molecules with better antibacterial and anti-enzymatic activities, prompted us to undertake the synthesis of this series of molecules. The presented study was to inaugurate new potent molecules against the two enzymes taken into account i.e. α-glucosidase and lipoxygenase enzymes. The attempt remained fruitful up to some extent because small number of molecules depicted inhibitory action against the both enzymes, as discussed in results and discussion section.

MATERIAL AND METHODS

General: 2,4-Dimethylaniline, 4-tosyl chloride and all alkyl/aralkyl halides were purchased from Alfa Aesar, Sigma Aldrick and Merck through local suppliers and were processed without further purification. The solvents used were of analytical grade. Reactions were monitored by pre-coated TLC silica gel G-25-UV_{254} plates using ethyl acetate and *n*-hexane as solvent system. Melting points were checked on Gallonkamp melting point apparatus by open capillary tube and were uncorrected. FTIR spectra were recorded on a MIDAC M 2000 spectrometer. ^{1}H-NMR spectra were recorded in $CHCl_3$-d_1 on Bruker spectrometer operating at 400 MHz at 25 ^{o}C. The chemical shifts are given in ppm and coupling constant in hertz (Hz). The abbreviations used in ^{1}H-NMR spectral interpretation were as, s = singlet, d = doublet, ddd = double doublet of doublet, t =triplet, q = quartet, qui = quintet, sex = sextet and sep = septet. Mass spectra (EIMS) were measured on Finnigan MAT-312 instrument along with data system.

Procedure for the synthesis of *N*-(2,4-Dimethylphenyl)-4-toluenesulfonamide (3): 2,4-Dimethylaniline (**1**; 0.05 mol) was suspended in 50 mL distilled water, followed by the addition of 10% aqueous Na_2CO_3 solution to make a pH of 8-10. The equimolar amount of 4-tosyl chloride (**2**; 0.05 mol) was added along with stirring gradually. The decrease in pH of reaction mixture was avoided by the addition of Na_2CO_3 solid at 25 ^{o}C. The reaction solution was stirred for further 3-4 hours. After complete reaction, indicated by TLC, a few drops of concentrated HCl were added slowly along with hand shaking. The reaction mixture was left undisturbed for 3-5 min. The light goldenrod yellow precipitates of title compound were collected by filtration, washed with distilled water and dried for further analysis. Light goldenrod yellow amorphous solid; Yield: 92%; M.P: 92.8 °C; Molecular formula: $C_{15}H_{17}NO_2S$; Molecular weight: 275 gmol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 3240 (N-H), 2980 (Ar C-H), 1600 (Ar C=C), 1430 (S=O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.57 (d, *J* = 8.4 Hz, 2H, H-2', H-6'), 7.19 (d, *J* = 8.0 Hz, 2H, H-3', H-5'), 7.10 (d, *J* = 8.4 Hz, 1H, H-6), 6.90 (d, *J* = 8.4 Hz, 1H, H-5), 6.88 (s, 1H, H-3), 6.10 (s, 1H, N-H), 2.37 (s, 3H, CH_3-2), 2.23 (s, 3H, CH_3-4'), 1.93 (s, 3H, CH_3-4); EIMS (*m/z*): 275 $[M]^+$, 211 $[C_{15}H_{17}N]^+$, 155 $[C_7H_7SO_2]^+$, 120 $[C_8H_{10}N]^+$, 105 $[C_8H_8]^+$, 91 $[C_7H_7]^+$.

General procedure for the synthesis of *N*-alkyl/aralkyl substituted sulfonamides (5a-o): The calculated amount of **3** (0.007 mol; 0.2 g) was taken in a 50 mL round bottom flask followed by the addition of 10 mL DMF to dissolve it. Lithium hydride (0.004 g; LiH) was also added to activate **3** for further reaction. The mixture was stirred for 30-45 minutes at 25 ^{o}C followed by the addition of alkyl/aralkyl halides (**4a-o**; 0.007 mol). The reaction contents were kept on stirring for 4-5 hours. The reaction was monitored by frequently performed TLC till single spot. After addition of ice cold water, the final products were collected by filtration or solvent extraction (using $CHCl_3$) depending upon the nature of product.

***N*-Ethyl-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (5a):** Yellowish brown amorphous sticky solid; Yield: 82%; Molecular formula: $C_{17}H_{21}NO_2S$; Molecular weight: 303 gmol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 2985 (Ar C-H), 1603 (Ar C=C), 1437 (S=O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.56 (d, *J* = 8.4 Hz, 2H, H-2', H-6'), 7.25 (d, *J* = 8.4 Hz, 2H, H-3', H-5'), 7.07 (s, 1H, H-3), 6.83 (d, *J* = 8.0 Hz, 1H, H-6), 6.43 (d, *J* = 8.0 Hz, 1H, H-5), 3.23 (q, *J* = 6.8 Hz, 2H, H-1''), 2.41 (s, 3H, CH_3-2), 2.31 (s, 3H, CH_3-4'), 2.28 (s, 3H, CH_3-4), 1.01 (t, *J* = 7.2 Hz, CH_3-2''); EIMS (*m/z*): 303 $[M]^+$, 210 $[C_{15}H_{16}N]^+$, 155 $[C_7H_7SO_2]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 29 $[C_2H_5]^+$.

***N*-Propyl-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (5b):** Transparent crystalline solid; Yield: 85%; M.P: 128 °C; Molecular formula: $C_{18}H_{23}NO_2S$; Molecular weight: 317 gmol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 2982 (Ar C-H), 1607 (Ar C=C), 1433 (S=O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.54 (d, *J* = 8.4 Hz, 2H, H-2', H-6'), 7.25 (d, *J* = 8.4 Hz, 2H, H-3', H-5'), 7.06 (s, 1H, H-3), 6.82 (d, *J* = 7.6 Hz, 1H, H-6),6.44 (d, *J* = 7.6 Hz, 1H, H-5), 3.65 (t, *J* = 7.6 Hz, 2H, H-1''), 3.12 (sex, *J* = 7.2 Hz, 2H, H-2''), 2.42 (s, 3H, CH_3-2), 2.31 (s, 3H, CH_3-4'), 2.27 (s, 3H, CH_3-4), 0.82 (t, *J* = 7.6 Hz, 3H, CH_3-3''); EIMS (*m/z*): 317 $[M]^+$, 210 $[C_{15}H_{16}N]^+$, 155 $[C_7H_7SO_2]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 43 $[C_3H_7]^+$.

***N*-(1-Methylethyl)-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (5c):** Milky white crystalline solid; Yield: 93%; M.P: 121.7 °C; Molecular formula: $C_{18}H_{23}NO_2S$; Molecular weight: 317 gmol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 2976 (Ar C-H), 1601 (Ar C=C), 1440 (S=O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.60 (d, *J* = 8.4 Hz, 2H, H-2', H-6'), 7.24 (d, *J* = 8.0 Hz, 2H, H-3', H-5'), 7.09 (s, 1H, H-3), 6.86 (d, *J* = 8.0 Hz, 1H, H-6), 6.59 (d, *J* = 8.0 Hz, 1H, H-5), 4.54 (sep, *J* = 6.8 Hz, 1H, H-1''), 2.40 (s, 3H, CH_3-2), 2.31 (s, 3H, CH_3-4'), 2.30 (s, 3H, CH_3-4), 1.04 (d, *J* = 6.8 Hz, 3H, CH_3-2''), 0.92 (d, *J* = 6.8 Hz, 3H, CH_3-3''); EIMS (*m/z*): 317 $[M]^+$, 210 $[C_{15}H_{16}N]^+$, 155 $[C_7H_7SO_2]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 43 $[C_3H_7]^+$.

***N*-Butyl-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (5d):** Bone white amorphous solid; Yield: 95%; M.P: 53.5 °C; Molecular formula: $C_{19}H_{25}NO_2S$; Molecular weight: 331 gmol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 2987 (Ar C-H), 1608 (Ar C=C), 1438 (S=O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.54 (d, *J* = 8.0 Hz, 2H, H-2', H-6'), 7.25 (d, *J* = 8.4 Hz, 2H, H-3', H-5'), 7.06 (s, 1H, H-3), 6.82 (d, *J* = 8.0 Hz, 1H, H-6), 6.43 (d, *J* = 8.4 Hz, 1H, H-5), 3.67 (ddd, *J* = 13.2, 4.0 Hz, 1H, H_a-1''), 3.13 (ddd, *J* = 13.2, 4.0 Hz, 1H, H_b-1''), 2.41 (s, 3H, CH_3-2), 2.31 (s, 3H, CH_3-4'), 2.28 (s, 3H, CH_3-4), 1.28-1.25 (m, 4H, H-2'', H-3''), 0.82 (t, *J* = 7.2 Hz, 3H, CH_3-4''); EIMS (*m/z*): 331 $[M]^+$, 210 $[C_{15}H_{16}N]^+$, 155 $[C_7H_7SO_2]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 57 $[C_4H_9]^+$.

***N*-(1-Methylpropyl)-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (5e):** White amorphous solid; Yield: 97%; M.P: 92.5 °C; Molecular formula: $C_{19}H_{25}NO_2S$; Molecular weight: 331 gmol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 2993 (Ar C-H), 1606 (Ar C=C), 1429 (S=O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.54 (d, *J* = 8.0 Hz, 2H, H-2', H-6'), 7.24 (d, *J* = 8.4 Hz, 2H, H-3', H-5'), 7.09 (s, 1H, H-3), 6.82 (d, *J* = 8.0 Hz, 1H, H-6), 6.43 (d, *J* = 8.4 Hz, 1H, H-5), 4.25 (sex, *J* = 7.2 Hz, 1H, H-1''), 2.41 (s, 3H, CH_3-2), 2.31 (s, 3H, CH_3-4'), 2.28 (s, 3H, CH_3-4), 1.01 (d, *J* = 6.8 Hz, 3H, CH_3-4''), 0.90-0.85 (m, 2H, H-2''), 0.74 (t, *J* = 7.2 Hz, 3H, CH_3-3''); EIMS (*m/z*): 331 $[M]^+$, 210 $[C_{15}H_{16}N]^+$, 155 $[C_7H_7SO_2]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 57 $[C_4H_9]^+$.

***N*-Pentyl-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (5f):** Colorless liquid; Yield: 65%; Molecular formula: $C_{20}H_{27}NO_2S$; Molecular weight: 345 gmol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 2974 (Ar C-H), 1604 (Ar C=C), 1435 (S=O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.54 (d, *J* = 8.4 Hz, 2H, H-2', H-6'), 7.25 (d, *J* = 8.0 Hz, 2H, H-3', H-5'), 7.06 (s, 1H, H-3), 6.82 (d, *J* = 8.0 Hz, 1H, H-6), 6.43 (d, *J* = 8.0 Hz, 1H, H-5), 3.65 (ddd, *J* = 12.8, 3.6 Hz, 1H, H_a-1''), 3.13 (ddd, *J* = 14.4, 4.4 Hz, 1H, H_b-1''), 2.41 (s, 3H, CH_3-2), 2.31 (s, 3H, CH_3-4'), 2.27 (s, 3H, CH_3-4), 1.34-1.16 (m, 6H, H-2'' to H-4''), 0.80 (t, *J* = 6.8 Hz, 3H, CH_3-5''); EIMS (*m/z*): 345 $[M]^+$, 210 $[C_{15}H_{16}N]^+$, 155 $[C_7H_7SO_2]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 71 $[C_5H_{11}]^+$.

***N*-(1-Methylbutyl)-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (5g):** Light brown amorphous sticky solid; Yield: 55%; Molecular formula: $C_{20}H_{27}NO_2S$; Molecular weight: 345 gmol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 2987 (Ar C-H), 1609 (Ar C=C), 1437 (S=O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.58 (d, *J* = 8.0 Hz, 2H, H-2', H-6'), 7.23 (d, *J* = 7.2 Hz, 2H, H-3', H-5'), 7.07 (d, *J* = 8.4 Hz, 1H, H-6), 6.91 (s, 1H, H-3), 6.86 (d, *J* = 8.4 Hz, 1H, H-5), 4.92-4.86 (m, 1H, H_a-1''), 4.29 (ddd, *J* = 13.6, 6.8 Hz, 1H, H_b-1''), 2.41 (s, 3H, CH_3-2), 2.31 (s, 3H, CH_3-4'), 2.27 (s, 3H, CH_3-4), 1.35-1.30 (m, 4H, H-2'', H-3''), 1.02 (d, *J* = 6.8 Hz, 3H, CH_3-5''), 0.88 (t, *J* = 8.4 Hz, 3H, CH_3-4''); EIMS (*m/z*): 345 $[M]^+$, 210 $[C_{15}H_{16}N]^+$, 155 $[C_7H_7SO_2]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 71 $[C_5H_{11}]^+$.

***N*-Heptyl-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (5h):** Flavescent amorphous solid; Yield: 65%; M.P: 54 °C; Molecular formula: $C_{22}H_{31}NO_2S$; Molecular weight: 373 gmol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 2977 (Ar C-H), 1605 (Ar C=C), 1445 (S=O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.54 (d, *J* = 8.0 Hz, 2H, H-2', H-6'), 7.24 (d, *J* = 8.0 Hz, 2H, H-3', H-5'), 7.01 (s, 1H, H-3), 6.92 (d, *J* = 8.0 Hz, 1H, H-6), 6.76 (d, *J* = 8.0 Hz, 1H, H-5), 3.67-3.64 (m, 1H, H_a-1''), 3.15-3.09 (m, 1H, H_b-1''), 2.44 (s, 3H, CH_3-2), 2.31 (s, 3H, CH_3-4'), 2.02-1.97 (m, 4H, H-2'', H-3''), 1.87 (s, 3H, CH_3-4), 1.25-1.18 (m, 6H, H-4'' to H-6''), 0.82 (t, *J* = 7.2 Hz, 3H, CH_3-7''); EIMS (*m/z*): 373 $[M]^+$, 210 $[C_{15}H_{16}N]^+$, 155 $[C_7H_7SO_2]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 99 $[C_7H_{15}]^+$, 91 $[C_7H_7]^+$.

***N*-Octyl-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (5i):** Light pink amorphous sticky solid; Yield: 65%; Molecular formula:

$C_{23}H_{33}NO_2S$; Molecular weight: 387 gmol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 2976 (Ar C-H), 1604 (Ar C=C), 1438 (S=O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.54 (d, *J* = 8.0 Hz, 2H, H-2', H-6'), 7.24 (d, *J* = 7.2 Hz, 2H, H-3', H-5'), 7.06 (s, 1H, H-3), 6.92 (d, *J* = 8.0 Hz, 1H, H-6), 6.75 (d, *J* = 8.0 Hz, 1H, H-5), 3.67-3.61 (m, 1H, H_a-1"), 3.16-3.10 (m, 1H, H_b-1"), 2.41 (s, 3H, CH_3-2), 2.31 (s, 3H, CH_3-4'), 2.02-1.97 (m, 6H, H-2" to H-4"), 1.86 (s, 3H, CH_3-4),1.24-1.17 (m, 6H, H-5" to H-7"), 0.83 (t, *J* = 7.2 Hz, 3H, CH_3-8"); EIMS (*m/z*): 387 $[M]^+$, 210 $[C_{15}H_{16}N]^+$, 155 $[C_7H_7SO_2]^+$, 119 $[C_8H_9N]^+$, 113 $[C_8H_{17}]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$.

***N*-Benzyl-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (5j):** Antique white crystalline solid; Yield: 85%; M.P: 85.2 °C; Molecular formula: $C_{22}H_{23}NO_2S$; Molecular weight: 365 gmol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 2970 (Ar C-H), 1610 (Ar C=C), 1439 (S=O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.60 (d, *J* = 8.4 Hz, 2H, H-2', H-6'), 7.28 (d, *J* = 8.4 Hz, 2H, H-3', H-5'), 7.18-7.16 (m, 3H, H-3" to H-5"), 7.12 (m, 2H, H-2", H-6"), 6.89 (s, 1H, H-3), 6.78 (d, *J* = 8.0 Hz, 1H, H-6), 6.46 (d, *J* = 8.0 Hz, 1H, H-5), 4.93 (d, *J* = 13.6 Hz, 1H, H_a-7"), 4.18 (d, *J* = 13.2 Hz, 1H, H_b-7"), 2.44 (s, 3H, CH_3-2), 2.22 (s, 3H, CH_3-4'), 1.94 (s, 3H, CH_3-4); EIMS (*m/z*): 365 $[M]^+$, 210 $[C_{15}H_{16}N]^+$, 155 $[C_7H_7SO_2]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 65 $[C_5H_5]^+$.

***N*-(2-Chlorobenzyl)-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (5k):** Blanched almond amorphous solid; Yield: 90%; M.P: 118 °C; Molecular formula: $C_{22}H_{22}ClNO_2S$; Molecular weight: 399 gmol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 2987 (Ar C-H), 1608 (Ar C=C), 1433 (S=O), 700 (C-Cl); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.60 (d, *J* = 8.0 Hz, 2H, H-2', H-6'), 7.36 (dd, *J* = 9.2, 3.6 Hz, 1H, H-3"), 7.28 (d, *J* = 8.0 Hz, 2H, H-3', H-5'), 7.19 (dd, *J* = 9.6, 4.0 Hz, 1H, H-6"), 7.12-7.10 (m, 2H, H-4", H-5"), 6.87 (s, 1H, H-3), 6.78 (d, *J* = 8.0 Hz, 1H, H-6), 6.55 (d, *J* = 8.0 Hz, 1H, H-5), 5.04 (d, *J* = 12.4 Hz, 1H, H_a-7"), 4.49 (d, *J* = 11.6 Hz, 1H, H_b-7"), 2.44 (s, 3H, CH_3-2), 2.21 (s, 3H, CH_3-4'), 1.98 (s, 3H, CH_3-4); EIMS (*m/z*): 401 $[M+2]^+$, 399 $[M]^+$, 210 $[C_{15}H_{16}N]^+$, 155 $[C_7H_7SO_2]^+$, 125 $[C_7H_6Cl]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 90 $[C_7H_6]^+$, 65 $[C_5H_5]^+$.

***N*-(4-Chlorobenzyl)-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (5l):** Cream white crystalline solid; Yield: 93%; M.P: 93 °C; Molecular formula: $C_{22}H_{22}ClNO_2S$; Molecular weight: 399 gmol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 2986 (Ar C-H), 1604 (Ar C=C), 1437 (S=O), 705 (C-Cl); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.79 (d, *J* = 8.4 Hz, 2H, H-2', H-6'), 7.58 (d, *J* = 8.0 Hz, 2H, H-3", H-5"), 7.15 (d, *J* = 8.0 Hz, 2H, H-3', H-5'), 7.05 (d, *J* = 8.0 Hz, 2H, H-2", H-6"), 6.91 (s, 1H, H-3), 6.76 (d, *J* = 8.0 Hz, 1H, H-6), 6.44 (d, *J* = 8.0 Hz, 1H, H-5), 4.89 (d, *J* = 13.6 Hz, 1H, H_a-7"), 4.15 (d, *J* = 13.2 Hz, 1H, H_b-7"), 2.44 (s, 3H, CH_3-2), 2.31 (s, 3H, CH_3-4'), 2.23 (s, 3H, CH_3-4); EIMS (*m/z*): 401 $[M+2]^+$, 399 $[M]^+$, 210 $[C_{15}H_{16}N]^+$, 155 $[C_7H_7SO_2]^+$, 125 $[C_7H_6Cl]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 90 $[C_7H_6]^+$, 65 $[C_5H_5]^+$.

***N*-(4-Bromobenzyl)-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (5m):** Champagne white crystalline solid; Yield: 89%; M.P: 120.2 °C; Molecular formula: $C_{22}H_{22}BrNO_2S$; Molecular weight: 443 gmol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 2978 (Ar C-H), 1603 (Ar C=C), 1428 (S=O), 653 (C-Br); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.58 (d, *J* = 8.4 Hz, 2H, H-2', H-6'), 7.30 (d, *J* = 8.4 Hz, 2H, H-3", H-5"), 7.28 (d, *J* = 8.0 Hz, 2H, H-3', H-5'), 7.00 (d, *J* = 8.4 Hz, 2H, H-2", H-6"), 6.91 (s, 1H, H-3), 6.79 (d, *J* = 8.0 Hz, 1H, H-6), 6.44 (d, *J* = 8.0 Hz, 1H, H-5), 4.86 (d, *J* = 13.6 Hz, 1H, H_a-7"), 4.14 (d, *J* = 13.6 Hz, 1H, H_b-7"), 2.44 (s, 3H, CH_3-2), 2.23 (s, 3H, CH_3-4'), 1.95 (s, 3H, CH_3-4); EIMS (*m/z*): 445 $[M+2]^+$, 443 $[M]^+$, 210 $[C_{15}H_{16}N]^+$, 171 $[C_7H_6Br]^+$, 155 $[C_7H_7SO_2]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 90 $[C_7H_6]^+$, 65 $[C_5H_5]^+$.

***N*-(2-Phenylethyl)-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (5n):** Shiny white crystalline solid; Yield: 87%; M.P: 91 °C; Molecular formula: $C_{23}H_{25}NO_2S$; Molecular weight: 379 gmol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 2989 (Ar C-H), 1609 (Ar C=C), 1443 (S=O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.58 (d, *J* = 8.4 Hz, 2H, H-2', H-6'), 7.28 (d, *J* = 8.0 Hz, 2H, H-3', H-5'), 7.22 (t, *J* = 7.6 Hz, 2H, H-3", H-5"), 7.17 (t, *J* = 6.8, 1H, H-4"), 7.06 (d, *J* = 7.2 Hz, 2H, H-2", H-6"), 6.91 (s, 1H, H-3), 6.79 (d, *J* = 8.0 Hz, 1H, H-6), 6.44 (d, *J* = 8.0 Hz, 1H, H-5), 2.85 (t, *J* = 6.8 Hz, 2H, H-8"), 2.64 (t, *J* = 6.8 Hz, 2H, H-7"), 2.44 (s, 3H, CH_3-2), 2.23 (s, 3H, CH_3-4'), 1.95 (s, 3H, CH_3-4); EIMS (*m/z*): 379 $[M]^+$, 210 $[C_{15}H_{16}N]^+$, 155 $[C_7H_7SO_2]^+$, 119 $[C_8H_9N]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 65 $[C_5H_5]^+$.

***N*-(3-Phenylpropyl)-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (5o):** Light brown amorphous solid; Yield: 90%; M.P: 70 °C; Molecular formula: $C_{24}H_{27}NO_2S$; Molecular weight: 393 gmol^{-1}; IR (KBr, ν_{max}, cm^{-1}): 2987 (Ar C-H), 1607 (Ar C=C), 1441 (S=O); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.52 (d, *J* = 8.0 Hz, 2H, H-2', H-6'), 7.24 (d, *J* = 7.6 Hz, 2H, H-3', H-5'), 7.21 (t, *J* = 7.6 Hz, 2H, H-3", H-5"), 7.15 (t, *J* = 7.2, 1H, H-4"), 7.07 (br.s, 2H, H-2",H-6"), 7.01 (s, 1H, H-3), 6.82 (d, *J* = 8.0 Hz, 1H, H-6), 6.44 (d, *J* = 8.0 Hz, 1H, H-5), 3.73-3.68 (m, 1H, H_a-9"), 3.22-3.18 (m, 1H, H_b-9"), 2.62-2.53 (m, 2H, H-7"), 2.41 (s, 3H, CH_3-2), 2.33 (s, 3H, CH_3-4'), 2.28 (s, 3H, CH_3-4), 2.00 (qui, *J* = 11.6 Hz, 2H, H-8"); EIMS (*m/z*): 393 $[M]^+$, 210

$[C_{15}H_{16}N]^+$, 155 $[C_7H_7SO_2]^+$, 119 $[C_8H_9N]^+$, 119 $[C_9H_{11}]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^+$, 65 $[C_5H_5]^+$.

α-Glucosidase assay: The α-glucosidase inhibition activity was performed according to the slightly modified method [16]. Total volume of the reaction mixture was 100 µL containing 70 µL of 50 mM phosphate buffer saline with pH of 6.8, 10 µL (0.5 mM) test compound and 10 µL (0.057 units) enzyme. The contents were mixed, pre-incubated for 10 min at 37 ºC and pre-read at 400 nm. The reaction was initiated by the addition of 10 µL of 0.5 mM substrate (p-nitrophenyl glucopyranoside). Acarbose was used as positive control. After 30 min of incubation at 37 ºC, absorbance was measured at 400 nm using Synergy HT microplate reader. All experiments were carried out in duplicates. The percent inhibition was calculated by the following equation:

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

IC_{50} values (concentration at which there is 50% in enzyme catalyzed reaction) compounds were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA). IC_{50} values were calculated (as mean of three independent experiments) from the graph by dilution of compounds to different concentrations.

Lipoxygenase activity: Lipoxygenase activity was assayed according to the method [17,18] with slight modifications. A total volume of 200 µL lipoxygenase assay mixture contained 150 µL sodium phosphate buffer (100 mM, pH 8.0), 10 µL test compound and 15µL purified lipoxygenase enzyme. The contents were mixed and preread at 234 nm and preincubated 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 $well^{-1}$) was used as a positive control. The percentage inhibition (%) was calculated by the same procedure as mentioned for α-glucosidase enzyme.

RESULTS AND DISCUSSION

N-substituted derivatives of *N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (**3**) were synthesized by the protocol depicted in scheme-1. The reaction procedures along with conditions are discussed in experimental section. The proposed structures of all the synthesized compounds were corroborated through spectral analysis using IR, ^{1}H-NMR and EIMS techniques. These molecules were further screened against α-glucosidase and lipoxygenase enzymes with an aim to introduce new potent molecules against these important enzymes in the field of pharmacology, as discussed in introduction.

Chemistry: The molecule, *N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (**3**) was synthesized through the reaction of 2,4-dimethylaniline (**1**) and 4-tosyl chloride (**2**) by a benignant method using distilled water as reaction medium. The product was acquired by acidification after 3-4 hours of stirring. Acidification by a dilute acid is essential for better yield but surplus amount has negative effect. The second step yielded the target *N*-substituted sulfonamides (**5a-o**) by the coupling of alkyl/aralkyl halides (**4a-o**) and **3** in a polar aprotic solvent like DMF and LiH as an activator. The activator removed the acidic proton first and then alkyl/aralkyl group is attached to nitrogen of sulfamoyl group. The sulfonamide **3** was obtained as light goldenrod yellow powder in a better yield. Its molecular formula was affirmed through EI-MS showing a $[M]^+$ ion peak at *m/z* 275 stepping to the molecular formula, $C_{15}H_{17}NO_2S$. The molecular formula was also confirmed by counting the number of protons using integration curves in its ^{1}H-NMR spectrum. In ^{1}H-NMR spectrum, the two doublets at δ 7.57 (d, J = 8.4 Hz, 2H, H-2', H-6'), 7.19 (d, J = 8.0 Hz, 2H, H-3', H-5') and one singlet at δ 2.23 (s, 3H, CH_3-4') were assigned to the seven protons of 4-tosyl group present in the molecule. Three signals resonating at δ 7.10 (d, J = 8.4 Hz, 1H, H-6), 6.90 (d, J = 8.4 Hz, 1H, H-5), 6.88 (s, 1H, H-3) with single intensity and two signals at δ 2.37 (s, 3H, CH_3-2) and 1.93 (s, 3H, CH_3-4) with triple intensity were allocated to the protons of dimethyl-substituted aniline ring. The IR spectrum supported by affirming the main functional groups present in the molecule by the absorption bands at 3240 (N-H), 2980 (Ar C-H), 1600 (Ar C=C) and 1430 (S=O). All these evidences corroborated the proposed structure of **3** and named as *N*-(2,4-dimethylphenyl)-4-toluenesulfonamide. The structures of other synthesized molecules (**5a-o**) were elucidated using the spectral data, as described in experimental section. The mass fragmentation pattern of the **5k** is provided in figure-1 for convenience to read out the other EIMS patterns.

In vitro biological activity: All the synthesized molecules, **3** and **5a-o**, were screened against the two enzymes; α-glucosidase and lipoxygenase and found to very moderate in their inhibitory action against the both ones. The results are presented as %age inhibition and IC_{50} values in table-1. The molecules, **5a**, **5b**, **5d** and **5f** exhibited moderate activity against the two enzymes but **5e**, **5g**, **5h**, **5n** and **5o** remained inactive. The most active

molecules was **5f** which executed the promising inhibitory action among the series of compounds, probably because of the presence of small medium sized aliphatic group attached to nitrogen of sulfamoyl group.

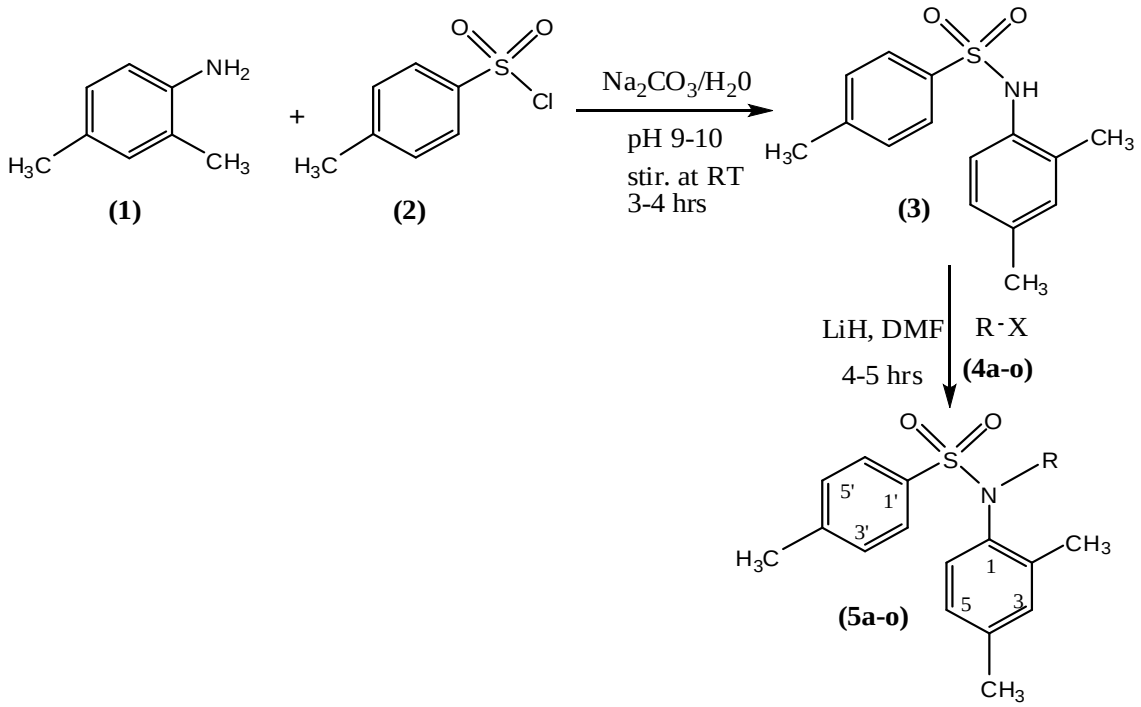
The screening of the synthesized molecules against α-glucosidase enzyme demonstrated that the some of them were active with moderate inhibitory potential as evident from their IC_{50} values (table-1). Among these molecules *N*-pentyl-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (**5f**), *N*-benzyl-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (**5j**) and *N*-(4-bromobenzyl)-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (**5m**) were found to be the most active inhibitors with their IC_{50} values of 184.73±1.46, 191.73±1.37 and 191.44±1.73 μmoles/L respectively, relative to acarbose a reference standard with IC_{50} value of 38.25±0.12 μmoles/L. These three molecules executed better activity among the series credibly due to small medium sized alkyl chain, unsubstituted aralkyl group and *p*-substituted halogenated aralkyl group respectively. The compounds, **5c** and **5l** showed least inhibitory values but **3**, **5e**, **5g**, **5h**, **5i**, **5n** and **5o** expressed no activity at all, as shown in table-1. Against lipoxygenase enzyme, the most of the synthesized molecules remained inactive but *N*-pentyl-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (**5f**) was found to be better inhibitor with their IC_{50} values of 191.76±0.97 μmoles/L respectively, relative to baicalein a reference standard with IC_{50} value 22.4±1.3 μmoles/L.

CONCLUSION

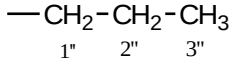
All the molecules were synthesized in excellent yields by a simple benign method, discussed in detail in experimental section. The structures of all molecules were well supported by their spectral data of IR, ^{1}H-NMR and EIMS. The enzyme inhibition results of all the synthesized compounds rendered them as very moderate inhibitors of α-glucosidase and lipoxygenase enzymes. The only molecule **5f** remained the most active inhibitor against the both enzymes. Some of them showed moderate inhibition but most remained inactive at all.

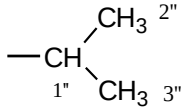
ACKNOWLEDGEMENTS

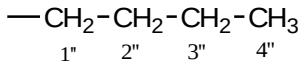
The authors are thankful to the Higher Education Commission (HEC) of Pakistan for the financial assistance.

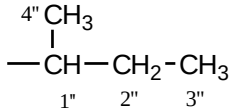


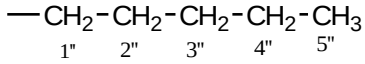
C. #	R	C. #	R
5a	—CH_2–CH_3 (1" 2")	**5i**	—CH_2–CH_2–CH_2–CH_2–CH_2–CH_2–CH_2–CH_3 (1" 2" 3" 4" 5" 6" 7" 8")

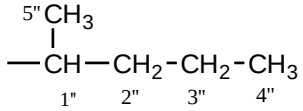
5b 

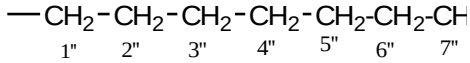
5c 

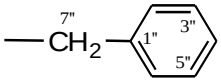
5d 

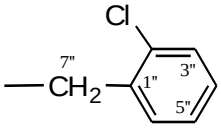
5e 

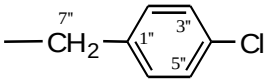
5f 

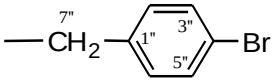
5g 

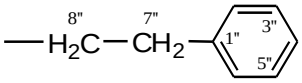
5h 

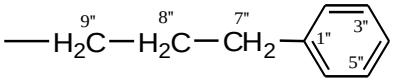
5j 

5k 

5l 

5m 

5n 

5o 

Scheme 1: *N*-substituted derivatives of *N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (**3**)

Table 1: Enzyme inhibition activity against α-glucosidase and lipoxygenase enzymes

Compound	α-Glucosidase enzyme		Lipoxygenase enzyme	
	%age Inhibition	IC_{50} (µM)	%age inhibition	IC_{50} (µM)
3	25.67±0.85	-	52.22±0.91	>400
5a	90.12±3.22	352.71±1.14	78.89±1.13	234.76±0.78
5b	88.63±3.55	222.15±1.21	77.04±1.33	239.97±0.76
5c	40.32±1.75	>500	53.83±0.78	>400
5d	95.26±3.82	211.72±1.92	67.65±1.01	313.42±0.95
5e	26.33±1.51	-	23.58±0.98	-
5f	94.31±1.12	184.73±1.46	95.19±1.25	191.76±0.97
5g	5.22±1.25	-	1.98±1.24	-
5h	10.31±1.31	-	49.39±0.68	-
5i	9.78±3.25	-	67.92±0.79	318.76±0.91
5j	92.37±3.64	191.73±1.37	23.45±0.87	-
5k	90.12±3.75	210.21±1.25	18.65±1.19	-
5l	40.63±1.75	>500	12.68±1.34	-
5m	84.63±1.16	191.44±1.73	23.29±0.71	-
5n	25.32±1.18	-	36.97±0.86	-
5o	4.78±2.12	-	3.53±0.96	-
Control	**92.23±0.14[a]**	**38.25±0.12[a]**	**93.79±1.27[b]**	**22.4±1.3[b]**

a = Acarbose **b** = Baicalein

Note: IC_{50} values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ–Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA). IC_{50} values were calculated (as mean of three independent experiments) from the graph by dilution of compounds to different concentrations.

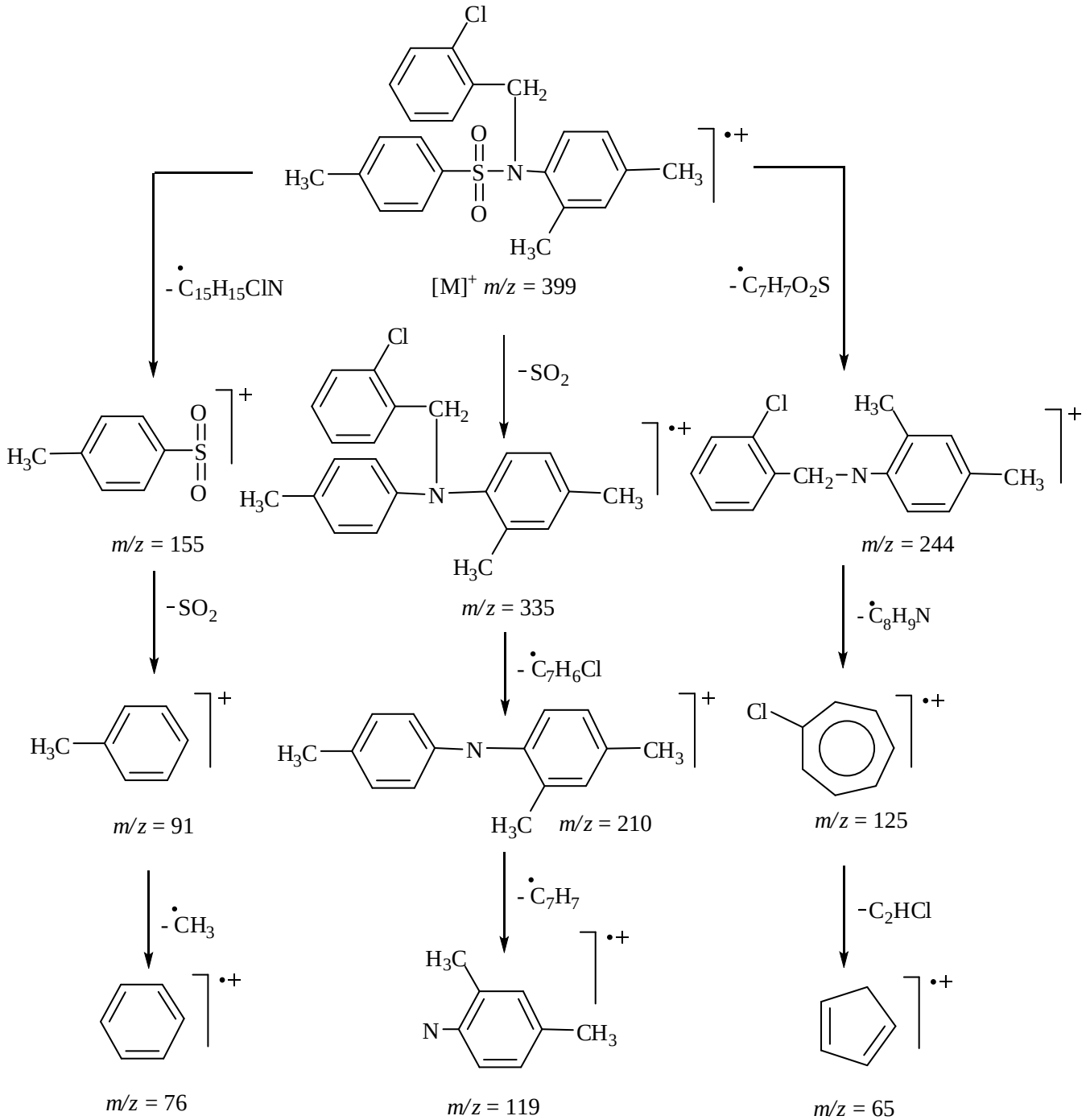


Figure-1: Mass Fragmentation pattern of *N*-(2-Chlorobenzyl)-*N*-(2,4-dimethylphenyl)-4-toluenesulfonamide (**5k**)

REFERENCES

1. Hansch C et al. Comprehensive Medicinal Chemistry, Pergamon Press: Oxford, **1990**.
2. Koesukwiwat U et al. Solid-phase extraction for multiresidue determination of sulfonamides, tetracyclines and pyrimethamine in Bovine's milk. J Chromatogr A 2007; 1149(1): 102-11.
3. Kleemann A et al. Pharmaceutical Substances: Synthesis, Patents and Applications, 2nd ed.; Thieme Stuggart Guide, **1999**.
4. Caballero RD et al. Micellar chromatographic procedure with direct injection for the determination of Sulfonamides in milk and honey samples. J Liq Chromatogr Rel Technol 2001; 24(1): 117-31.

5. (a) Roush WR et al. Vinyl sulfonate esters and vinyl sulfonamides: Potent, irreversible inhibitors of Cysteine Proteases. J Am Chem Soc 1998; 120(42): 10994-5. (b) Roush WR et al. Potent second generation vinyl sulfonamide inhibitors of the trypanosomal cysteine protease cruzain. Bioorg Med Chem Lett 2001; 11(20): 2759-62.
6. (a) Caddick S et al. A new route to sulfonamides via intermolecular radical addition to pentafluorophenyl vinylsulfonate and subsequent aminolysis. Org Lett 2002; 4(15): 2549-51. (b) Caddick S et al. Solid-phase intermolecular radical reactions 2: Synthesis of *C*-Glycopeptide Mimetics *via* a novel acrylate acceptor. Org Lett 2002; 4(15): 1775-7.
7. Wyman P et al. Sulfonamide derivatives, process for their preparation and use as medicaments. US Patent 6,423,717, July 23, **2002**.
8. Rieder MJ et al. Time-course of toxicity of reactive sulfonamide metabolites. Toxicology 1995; 95(1-3): 141-6.
9. Chiba S. Molecular mechanism of α-glucosidase and glucoamylase. Biosci Biotech Bioch 1997; 61(8): 1233-9.
10. Baron AD. Postprandial hyperglycemia and alpha-glucosidase inhibitor. Diabetes Res Clin Pr 1998; 40(Suppl.): 51-5.
11. Lebovitz HE. Alpha-glucosidase inhibitors. Endocrinol Metab Clin North Am 1997; 26(3): 539-51.
12. (a) Clapp HC et al. Inhibition of soybean lipoxygenase 1 by *N*-alkylhydroxylamines. J Biochem 1985; 24(8): 1826-30. (b) Kemal C et al. Reproductive inactivation of soybean lipoxygenase activity. J Biochem 1987; 26(22): 7064-72.
13. (a) Steinhilber D. 5-Lipoxygenase: a target for anti-inflammatory drugs revisited. Curr Med Chem 1999; 6(1): 71-85. (b) Alitonou GA et al. Investigations on the essential oil of *Cymbopogon giganteus* from benin for its potential use as an anti-inflammatory agent. Int J Aromather 2006; 16(1): 37-41.
14. Abbasi MA et al. Synthesis structural characterization and biological screening of various sulfa drugs derived from 2-anisidine. J Chem Soc Pak 2013; 35(2): 404-10.
15. Aziz-ur-Rehman et al. Synthesis, characterization and biological screening of some 4-*O*-substituted derivatives of *N*-(4-hydroxyphenyl)-*N*-methyl-4-methylbenzenesulfonamide. Asian J Pharm Bio Res 2012; 2(2): 100-5.
16. Chapdelaine P et al. *p*-Nitrophenol-α-D-Glucopyranoside as substrate for measurement of maltase activity in Human Semen. Clin Chem 1978; 24(2): 208-11.
17. Tappel AL. The mechanism of the oxidation of unsaturated fatty acid catalyzed by hematin compounds. Arch Biochem Biophys 1953; 44(2): 378-95.
18. Baylac S, Racine P. Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts. Int J Aromather 2003; 13(2/3): 138-42.