Synthesis of some new biologically active *N*-substituted-2''-[(phenylsulfonyl)(piperidin-1-yl)amino]acetamide derivatives

Hira Khalid[1], Aziz-ur-Rehman[1]*, Muhammad Athar Abbasi[1], Sabahat Zahra Siddiqui[1], Abdul Malik[2], Muhammad Ashraf[3], Irshad Ahmad[4] and Syeda Abida Ejaz[4]

[1]Department of Chemistry, Government College University, Lahore, Pakistan
[2]HEJ Research Institute of Chemistry, ICCBS, University of Karachi, Karachi, Pakistan
[3]Department of Biochemistry and Biotechnology, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[4]Department of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

Abstract: A new series of *N*-aryl/aralkyl substitued-2''-[(phenylsulfonyl)(piperidin-1-yl)amino]acetamide **(7a-k)** was synthesized. These derivatives were geared up by the pairing of benzenesulfonyl chloride **(4)** with 1-aminopiperidine **(5)** under dynamic pH control in aqueous media to afford parent compound *N*-(Piperidin-1-yl) benzenesulfonamide **(6)**, followed by the substitution at nitrogen atom with different electrophiles *N*-aryl/aralkyl-substituted-2-bromoacetamides **(3a-k)** in the presence of sodium hydride (NaH) and *N,N*-Dimethylformamide (DMF) to give a new series of *N*-substituted derivatives of acetamide **(7a-k)** bearing piperidine moiety. All the synthesized compounds were confirmed on the basis of IR, EIMS and ^{1}H-NMR spectral data. The synthesized compounds were evaluated against acetylcholinesterase and butyrylcholinesterase (AChE and BChE) respectively and lipoxygenase (LOX) enzymes. Almost all the synthesized compounds displayed promising activity but few of them remained inactive against lipoxygenase enzymes.

Keywords: 1-Aminopiperidine; benzenesulfonyl chloride; acetamide; butyrylcholinesterase; spectral characterization.

INTRODUCTION

The piperidine nucleus is incorporated into numerous biologically dynamic compounds & secondary metabolites such as (*S*)-pipecolic acid related to brain disorder (Sanchez-Sancho *et al.*, 1998; Nithiya *et al.*, 2011; Adger *et al.*, 1996; Daly *et al.*, 1986). Compounds bearing piperidine moiety have various diligences in commercial & medicinal area. Effect of compounds bearing piperidine and pyrrolidine ring were assessed for plasma glucose level (Kozikowski *et al.*, 1998), insulin normalization and treatment of cocaine abuse (Brau *et al.*, 2000). Sulfonamides are well known for their potent enzyme inhibition activities (Supuran *et al.*, 2003). On the basis of these facts, a number of researchers are attempting to inaugurate new drugs with improved pharmacokinetics with less inauspicious effects.

Most of the drugs employed for the treatment of Alzheimer's disease are acetylcholinesterase inhibitors. Acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8) belong to the serine hydrolases enzyme family. The different specificities for the substrates and inhibitors for these enzymes are due to the variation in amino acid residues of the active sites of AChE and BChE. The enzyme system is dependable for the termination of acetylcholine at cholinergic synapses. These are key components of cholinergic brain synapses and neuromuscular junctions. The major role of the both enzymes is to catalyze the hydrolysis of the neurotransmitter acetylcholine and termination of the nerve impulse (Cygler *et al.*, 1993; Tougu, 2001). Thus, the search for novel cholinesterase inhibitors is a good strategy to inaugurate new drug candidates for Alzheimer's disease and related ailments (Gauthier, 2001; Bertaccini, 1982). Lipoxygenase enzymes have iron in their structural framework and are involved in dioxygenation of lipids, consisting of polyunsaturated fatty acids. It performs a key role in the synthesis of leukotrienes, which are responsible for pathophysiology of different allergic diseases. Lipoxygenase inhibitors are mainly used for the treatment of allergic and inflammatory diseases (Roussaki *et al.*, 2010; Aziz-ur-Rehman *et al.*, 2004).

In continuation of our previous work (Aziz-ur-Rehman *et al.*, 2011; Aziz-ur-Rehman *et al.*, 2012). The synthesis and biological activity of new *N*-aryl/aralkyl substitued-2''-[(phenylsulfonyl) (piperidin-1-yl) amino] acetamide compounds with an objective to detect the enzyme inhibition activity of the synthesized compounds. Here, we give an account of the synthesis of sulfonamides bearing piperidine moiety and acetamide functionality, with significant inhibitory potential against cholinesterase enzymes.

MATERIALS AND METHODS

General

Melting points of all the synthesized compounds were recorded on a Griffin and George apparatus by open

**Corresponding author:* e-mail: azizryk@yahoo.com

capillary tube and are uncorrected. Purity of the compounds was checked by thin layer chromatography (TLC) with solvent systems consisting of varying concentrations of EtOAc and *n*-hexane on aluminum sheets precoated with silica gel 60 F_{254} (20 x 20 cm, 0.2 mm thick; E-Merck). Visualization of the TLC plates was carried out under UV at 254 and 366 nm and also by spraying with ceric sulfate solution (with heating). The I.R. spectra were recorded in KBr pellet method on a Jasco-320 spectrophotometer (wave number in cm^{-1}). Proton NMR spectra were recorded in CD_3OD on a Bruker spectrometers operating at 300 MHz. Chemical shifts are given in ppm. EIMS were recorded on a JMS-HX-110 spectrometer, with a data system. 1-Aminopiperidine, benzenesulfonyl chloride, bromoacetyl bromide, substituted/unsubstituted aromatic amines and the other electrophilic reagents were purchased from Alfa Aesar & Sigma Aldrich through local suppliers. Other analytical grade solvents used were obtained from commercial suppliers.

General procedure for the synthesis of different N-aryl/aralkyl substituted-2-bromoacetamides (3a-k)

The calculated amount of substituted aryl/aralkyl amines **(1a-k)**; (15.0 mmoles) was taken in an iodine flask containing 15.0 mL of distilled water and 10% Na_2CO_3 solution was added to adjust the pH 9.0 to 10. The bromoacetyl bromide **(2)**; (15.0 mmoles) was then added drop wise in the reaction mass in 2-5 min. After complete addition, the iodine flask was vigorously shaken (manually) till the solid precipitates formed. The solid precipitates were further stirred for 40 min. The progress of reaction was monitored by thin layer chromatography (TLC) (*n*-hexane: ethyl acetate; 75: 25). After complete conversion, the obtained solid was filtered, washed with distilled water and dried to yield the corresponding electrophile *N*-aryl/aralkyl-substituted-2-bromoacetamide **(3a-k)**.

Procedure for the synthesis of N-(Piperidin-1-yl) benzenesulfonamide (6)

1-Aminopiperidine (2.6 mL, 10.0 mmol); **(5)** was suspended in 50.0 mL water and the pH was maintained at 9.0 by adding basic aqueous solution of Na_2CO_3 at 0-5^oC.Then benzenesulfonyl chloride (2.9mL, 10.0 mmol); **(4)** was added slowly. The reaction mixture was stirred at room temperature and monitored with TLC until completion. Concentrated HCl was added to adjust acidity of the reaction mixture to pH 2-3. The precipitation was then filtered and washed with distilled H_2O to give the title compound **(6)** on drying.

General procedure for the synthesis of N-aryl/aralkyl-substituted-2''-[(phenylsulfonyl)(piperidin-1-yl) amino]acetamide (7a-k)

To a solution of compound **(6)** (0.1 g, 0.40 mmol) in DMF (5 mL) was added NaH (0.01 g, 0.40 mmol) in small portions over 2-5 min at 0-5°C. Then, the reaction mixture was stirred for 15 min at room temperature. The corresponding *N*-substituted aryl/aralkyl-2-bromo-acetamide **(3a-k)**; (0.09g, 0.40 mmol) was added into the reaction mixture slowly and stirred for 10-15 min. The reaction mixture was then heated to 50 ^oC and stirred at this temperature for 30-40 min while monitoring the progress of the reaction by TLC. On completion of reaction, the reaction mixture was cooled to room temperature and quenched with cold water (50 mL). The settled precipitates were filtered, washed with water and dried to acquire the resultant derivatives. In some cases where no solid precipitation occurred, the product was obtained through solvent extraction method using chloroform to yield the corresponding *N*-aryl/aralkyl-substituted-2"-[(phenylsulfonyl) (piperidin-1-yl) amino] acetamide **(7a–k)** derivatives.

Spectral characterization of the synthesized compounds

N-(Piperidin-1-yl)benzenesulfonamide (6)

IR (KBr, cm^{-1}) ν_{max}: 3430 (N-H stretching), 3024 (C-H stretching of aromatic ring), 1546 (C=C stretching of aromatic ring), 1341 (-SO_2- stretching);^{1}H-NMR (300 MHz, CD_3OD,δ / ppm): 7.90 (dd, *J*=8.7,1.5Hz,2H, H-2'& H-6'), 7.65 (m, 2H, H-3'& H-5'), 7.52 (m, 1H, H-4'), 2.96 (t, *J*=5.4Hz, 2H, H_{eq}-2 & H_{eq}-6),2.47 (t, *J*=5.4Hz, 2H, H_{ax}-2 & H_{ax}-6), 1.61 (m,2H, CH_2-4),1.44 (m,4H, CH_2-3 &CH_2-5);EIMS *m/z*: 240 (24%) $[M]^+$, 176 (37%), 156 (54%), 141 (100%).

N-(4'''-Methoxyphenyl)-2''-[(phenylsulfonyl) (piperidin-1-yl) amino] acetamide (7a)

IR (KBr, cm^{-1}) ν_{max}: 3439 (N-H stretching), 3015 (C-H stretching of aromatic ring), 2925 (-CH_2- stretching), 1531 (C=C stretching of aromatic ring), 1329 (-SO_2- stretching); ^{1}H-NMR (300 MHz, CD_3OD,δ/ppm): 7.80 (dd, *J*=6.6, 1.8 Hz, 2H, H-2' & H-6'), 7.65 (m, 2H, H-3' & H-5'), 7.28 (m, 1H, H-4'),6.85 (d, *J*=9.0 Hz, 2H, H-2" & H-6"), 6.67 (d, *J*=8.7 Hz, 2H, H-3" & H-5"), 4.07 (s, 2H, CH_2-2"), 3.91 (s, 3H, H_3CO-4"), 2.94 (t, *J* = 5.4 Hz, 2H, H_{eq}-2 & H_{eq}-6),2.47 (t, *J*=5.4 Hz, 2H, H_{ax}-2 & H_{ax}-6), 1.70 (m, 2H, CH_2-4),1.58 (m,4H CH_2-3 & CH_2-5); EIMS *m/z*: 401 (17%) $[M]^+$, 372 (28%), 281 (51%), 239 (54%), 141 (100%).

N-Methyl-N- (4'''-hydroxyphenyl)-2''-[(phenylsulfonyl) (piperidin-1-yl) amino] acetamide (7b)

IR (KBr, cm^{-1}) ν_{max}: 3029 (C-H stretching of aromatic ring), 2940 (-CH_2- stretching), 1537 (C=C stretching of aromatic ring), 1319 (-SO_2-stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.79 (dd, *J*=6.6, 1.8 Hz, 2H, H-2' & H-6'), 7.62 (m, 2H, H-3' & H-5'),7.30 (m, 1H, H-4'), 6.91 (d, *J*=9.0 Hz, 2H, H-2" &H-6"), 6.71 (d, *J*=8.7 Hz, 2H, H-3" & H-5"), 4.13 (s,2H, CH_2-2"), 3.17 (s, 3H, CH_3-7), 2.93 (t, *J*=5.4 Hz, 2H, H_{eq}-2 & H_{eq}-6), 2.45 (t, *J*=5.4 Hz, 2H, H_{ax}-2 & H_{ax}-6), 1.69 (m, 2H, CH_2-4),1.55 (m,4H, CH_2-3 & CH_2-5); EIMS *m/z*: 403 (13%) $[M]^+$, 339 (27%), 281 (57%), 239 (71%), 93 (100%).

N-Benzyl-2''-[(phenylsulfonyl)(piperidin-1-yl) amino] acetamide (7c)

IR (KBr, cm^{-1}) ν_{max}: 3431 (N-H stretching), 3018 (C-H stretching of aromatic ring), 2945 (-CH_2- stretching), 1529 (C=C stretching of aromatic ring), 1323 (-SO_2- stretching); ^{1}H-NMR (300 MHz, CD_3OD,δ/ppm): 7.90 (dd, *J*=6.7,1.5Hz, 2H, H-2'&H-6'), 7.60-7.63 (m, 5H, H-2''' to H-6'''), 7.54 (m, 2H, H-3' &H-5'), 7.27 (m, 1H, H-4'), 4.35 (s, 2H, CH_2-2''),3.24 (s,2H, CH_2-7'''),2.95 (t, *J*=5.4Hz, 2H, H_{eq}-2 & H_{eq}-6),2.47 (t, *J*=5.4Hz, 2H, H_{ax}-2 & H_{ax}-6), 1.61(m, 2H, CH_2-4),1.56 (m,4H, CH_2-3 & CH_2-5); EIMS *m/z*: 387 (23%) $[M]^+$, 323 (45%), 303 (59%), 281 (73%), 91 (100%).

N-(2-Phenylethyl)-2''-[(phenylsulfonyl)(piperidin-1-yl) amino] acetamide (7d)

IR (KBr, cm^{-1}) ν_{max}: 3437 (N-H stretching), 3023 (C-H stretching of aromatic ring), 2932 (-CH_2- stretching), 1521 (C=C stretching of aromatic ring), 1327 (-SO_2- stretching); ^{1}H-NMR (300 MHz, CD_3OD,δ/ppm): 7.91 (dd, *J*=6.7,1.5Hz, 2H, H-2'& H-6'), 7.53 (m, 2H, H-3' & H-5'), 7.19-7.26 (m, 5H, H-2" to H-6"), 7.26 (m, 1H, H-4'),4.33 (s, 2H, CH_2-2''), 3.22 (t, *J*=6.5 Hz, 2H, CH_2-8"), 2.94 (t, *J*=5.4Hz, 2H, H_{eq}-2 & H_{eq}-6), 2.76 (t, *J*=6.5 Hz, 2H, CH_2-7"),2.48 (t, *J*=5.4Hz, 2H, H_{ax}-2 & H_{ax}-6), 1.59(m, 2H, CH_2-4),1.53(m,4H, CH_2-3 & CH_2-5); EIMS *m/z*: 401 (17%) $[M]^+$, 337 (31%), 317 (69%), 105 (84%), 91 (100%).

N-(2'''-Methoxy-5'''-chlorophenyl)-2''-[(phenylsulfonyl) (piperidin-1-yl)amino]acetamide (7e)

IR (KBr, cm^{-1}) ν_{max}: 3429 (N-H stretching), 3019 (C-H stretching of aromatic ring), 2943 (-CH_2- stretching), 1535 (C=C stretching of aromatic ring), 1325 (-SO_2- stretching); ^{1}H-NMR (300 MHz, CD_3OD,δ / ppm): 8.32 (d, *J*=2.4Hz, 1H, H-6'''), 7. 89 (dd, *J*=7.2, 1.5Hz, 2H, H-2', H-6'), 7.73 (m, 2H, H-3 , H-5'), 7.61 (m, 1H, H-4'), 7.01 (dd, *J*=8.7, 2.1 Hz, 1H, H-4'''), 6.94 (d, *J*=8.7Hz, 1H, H-3'''), 3.90 (s, 2H, CH_2-2''), 2.95 (t, *J*=5.4Hz, 2H, H_{eq}-2 & H_{eq}-6), 2.47 (t, *J*=5.4Hz, 2H, H_{ax}-2 & H_{ax}-6), 1.61 (m, 2H, CH_2-4), 1.44(m, 4H, CH_2-3 & CH_2-5); EIMS *m/z*: 437 (13%) $[M]^+$, 373 (25%), 353 (59%), 281 (73%), 141 (100%).

N-(2''',3'''-Dimethylphenyl)-2''-[(phenylsulfonyl) (piperidin-1-yl) amino] acetamide (7f)

IR (KBr, cm^{-1}) ν_{max}: 3440 (N-H stretching), 3032 (C-H stretching of aromatic ring), 2941 (-CH_2- stretching), 1523 (C=C stretching of aromatic ring), 1318 (-SO_2- stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.98 (dd, *J*=8.7,1.5Hz, 2H, H-2'& H-6'), 7.73 (m, 2H, H-3' & H-5'), 7.61 (m, 1H, H-4'), 7.32 (d, *J*=7.5, 1H, H-6'''), 7.05-7.10 (m, 2H, H-4'''& H-5'''), 4.02 (s, 2H, CH_2-2''), 3.14 (t, *J*=5.4Hz, 2H, H_{eq}-2 & H_{eq}-6), 2.47 (t, *J*=5.4Hz, 2H, H_{ax}-2 & H_{ax}-6), 2.28 (s, 3H, CH_3-3'''), 2.14 (s, 3H, CH_3-2'''), 1.71(m, 2H, CH_2-4), 1.56 (m, 4H, CH_2-3 & CH_2-5); EIMS *m/z*: 401 (15%) $[M]^+$, 337 (19%), 317 (43%), 141 (100%), 105 (61%).

N-(2''',4'''-Dimethylphenyl)-2''-[(phenylsulfonyl) (piperidin-1-yl) amino] acetamide (7g)

IR (KBr, cm^{-1}) ν_{max}: 3438 (N-H stretching), 3029 (C-H stretching of aromatic ring), 2939 (-CH_2- stretching), 1521 (C=C stretching of aromatic ring), 1315 (-SO_2- stretching); ^{1}H-NMR (300 MHz, CD_3OD, δ/ppm): 7.97 (dd, *J*=7.2,1.5Hz, 2H, H-2'& H-6'), 7.71 (m, 2H, H-3' & H-5'), 7.61 (m, 1H, H-4'), 7.43 (d, *J*=8.1Hz, 1H, H-6'''), 7.10 (d, *J*=8.1Hz, 1H, H-5'''), 6.78 (d, *J*=1.5, 1H, H-3'''), 4.10 (s, 2H, CH_2-2''), 2.99 (t, *J*=5.4Hz, 2H, H_{eq}-2 & H_{eq}-6),2.49 (t, *J*=5.4Hz, 2H, H_{ax}-2& H_{ax}-6), 1.61(m, 2H, CH_2-4),1.44 (m,4H, CH_2-3 & CH_2-5); EIMS *m/z*: 401 (13%) $[M]^+$, 337 (21%), 317 (31%), 141 (100%), 105 (58%).

N-(2''',5'''-Dimethylphenyl)-2''-[(phenylsulfonyl) (piperidin-1-yl) amino] acetamide (7h)

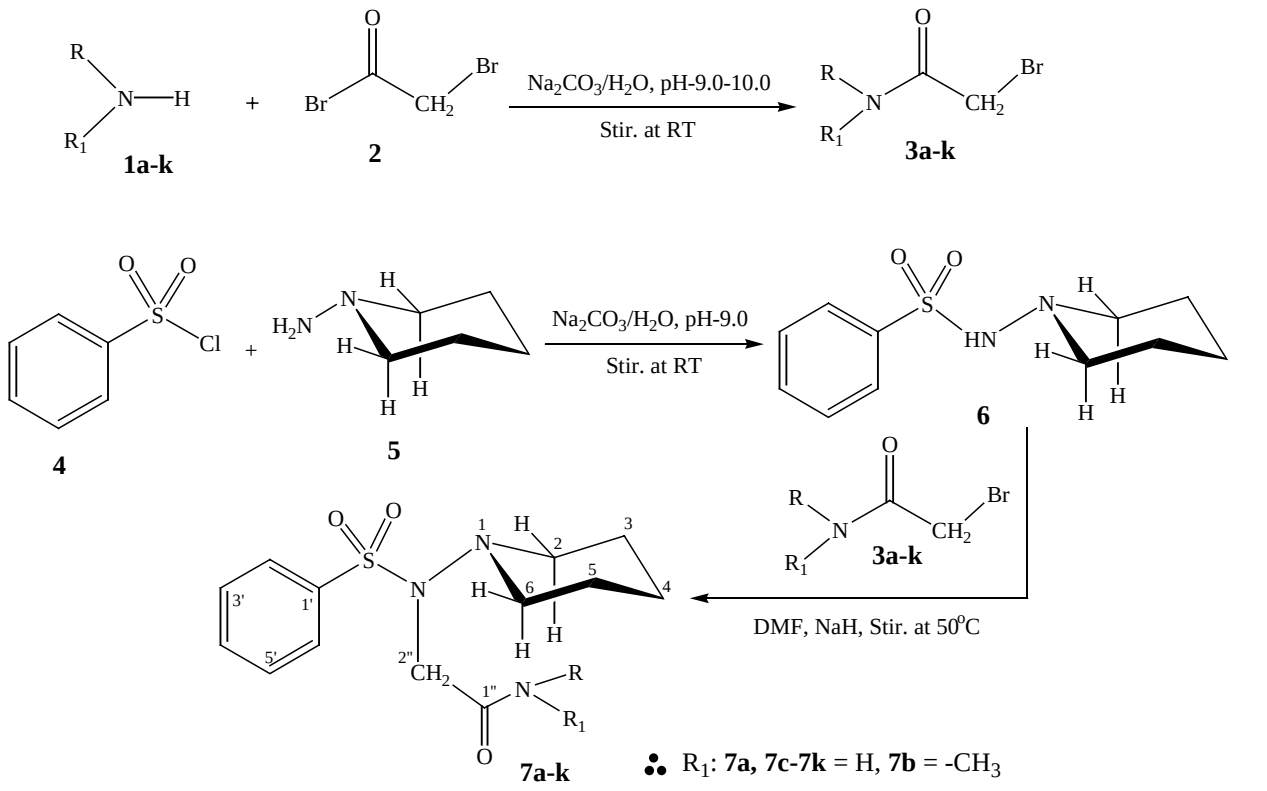
IR (KBr, cm^{-1}) ν_{max}: 3448 (N-H stretching), 3029 (C-H stretching of aromatic ring), 2935 (-CH_2- stretching), 1519 (C=C stretching of aromatic ring), 1313 (-SO_2- stretching); ^{1}H-NMR (300 MHz, CD_3OD,δ/ppm): 8.01 (d, *J*=1.5 Hz, 1H, H-6'''), 7.73 (dd, *J*=8.7,1.5Hz, 2H, H-2' & H-6'), 7.58 (m, 2H, H-3' &H-5'), 7.08 (m, 1H, H-4'), 6.91 (d, *J*=7.5Hz,1H, H-4'''),6.87 (d, *J*=7.5 Hz, 1H, H-3'''),4.17 (s, 2H, CH_2-2''),2.96 (t, *J*=5.4Hz, 2H, H_{eq}-2 & H_{eq}-6),2.47 (t, *J*=5.4Hz, 2H, H_{ax}-2 & H_{ax}-6),2.20 (s, 6H, CH_3-2''' & CH_3-5'''), 3.62 (s, 2H, CH_2-2''),1.59(m, 2H, CH_2-4), 1.41(m,4H, CH_2-3 & CH_2-5); EIMS *m/z*: 401 (19%) $[M]^+$, 337 (23%), 317 (35%), 141 (100%), 105 (59%).

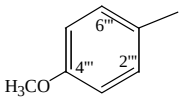
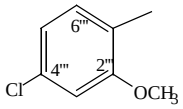
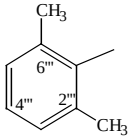
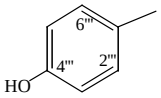
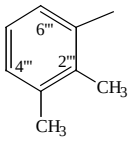
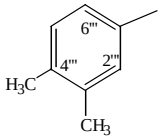
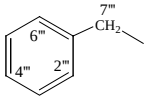
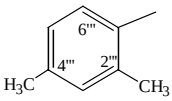
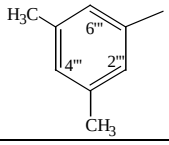
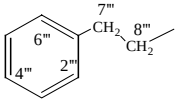
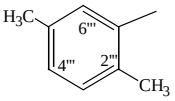
N-(2''',6'''-Dimethylphenyl)-2''-[(phenylsulfonyl) (piperidin-1-yl)amino]acetamide (7i)

IR (KBr, cm^{-1}) ν_{max}: 3441 (N-H stretching), 3033 (C-H stretching of aromatic ring), 2942 (-CH_2- stretching), 1521 (C=C stretching of aromatic ring), 1319 (-SO_2- stretching); ^{1}H-NMR (300 MHz, CD_3OD,δ/ppm): 7.97 (dd, *J*=8.7,1.5Hz, 2H, H-2'& H-6'), 7.73 (m, 2H, H-3' & H-5'), 7.61 (m, 1H H-4'), 7.06 (m, 3H, H-3''' to H-5'''), 2.96 (t, *J*=5.4Hz, 2H, H_{eq}-2 & H_{eq}-6), 2.47 (t, *J*=5.4Hz, 2H, H_{ax}-2 &H_{ax}-6),2.20 (s, 6H, CH_3-2''' & CH_3-6'''), 2.16 (s, 2H, CH_2-2''), 1.57(m, 2H, CH_2-4),1.42(m,4H, CH_2-3 & CH_2-5); EIMS *m/z*: 401 (23%) $[M]^+$, 337 (45%), 317 (21%), 141 (100%), 105 (45%).

N-(3''',4'''-Dimethylphenyl)-2''-[(phenylsulfonyl) (piperidin-1-yl)amino]acetamide (7j)

IR (KBr, cm^{-1}) ν_{max}: 3437 (N-H stretching), 3028 (C-H stretching of aromatic ring), 2937 (-CH_2- stretching), 1520 (C=C stretching of aromatic ring), 1313 (-SO_2- stretching); ^{1}H-NMR (300 MHz, CD_3OD,δ/ppm): 7.73 (dd, *J*=6.6,1.8Hz, 2H, H-2'& H-6'), 7.63 (m, 2H, H-3' &H-5'), 7.30 (m, 1H, H-4'),7.16 (d, *J*=8.1Hz, 2H, H-6'''), 7.02 (d, *J*=8.1Hz, 1H, H-5'''), 6.76 (s, 1H, H-2 "), 3.99 (s, 2H, CH_2-2''), 2.96 (t, *J*=5.4Hz, 2H, H_{eq}-2 & H_{eq}-6),2.47 (t, *J*=5.4Hz, 2H, H_{ax}-2& H_{ax}-6),2.23 (s, 3H, CH_3-



Compound	R	Compound	R	Compound	R
7a		**7e**		**7i**	
7b		**7f**		**7j**	
7c		**7g**		**7k**	
7d		**7h**			

Scheme 1: Outline for the synthesis of *N*-substituted acetamide bearing piperidine nucleus.

3'''), 2.20 (s, 3H, CH_3-4'''), 1.62(m, 2H, CH_2-4),1.45(m,4H, CH_2-3 & CH_2-5); EIMS *m/z*: 401 (13%) $[M]^+$, 337 (35%), 317 (44%), 141 (100%), 105 (31%).

N-(3''',5'''-Dimethylphenyl)-2''-[(phenylsulfonyl) (piperidin-1-yl)amino]acetamide (7k)

IR (KBr, cm^{-1}) ν_{max}: 3437 (N-H stretching), 3021 (C-H stretching of aromatic ring), 2931 (-CH_2- stretching), 1511 (C=C stretching of aromatic ring), 1311 (-SO_2- stretching);^{1}H-NMR (300 MHz, CD_3OD,δ/ppm): 7.73 (dd, *J*=6.6,1.8Hz, 2H, H-2'&H-6'), 7.63 (m, 2H, H-3' &H-5'), 7.22 (s, 2H, H-2''' & H-6'''), 6.76 (s, 1H, H-4'''), 4.01 (s, 2H, CH_2-2''), 2.96 (t, *J*=5.4Hz, 2H, H_{eq}-2& H_{eq}-6), 2.47 (t, *J*=5.4Hz, 2H, H_{ax}-2 & H_{ax}-6),2.26 (s, 6H, CH_3-3'''& CH_3-5'''), 1.69 (m, 2H, CH_2-4),1.41(m,4H, CH_2-3 & CH_2-5); EIMS *m/z*: 401 (10%) $[M]^+$, 337 (20%), 317 (34%), 141 (100%), 105 (51%).

Table 1: Physical data of the synthesized compounds

Samples	Appearance	Melting point (°C)	Molecular formula	%Yield
6	Light yellow powder	55-57	$C_{11}H_{16}N_2O_2S$	90
7a	Brown sticky solid	-	$C_{20}H_{25}N_3O_4S$	68
7b	Rust sticky solid	-	$C_{20}H_{25}N_3O_4S$	87
7c	Mustard sticky solid	-	$C_{20}H_{25}N_3O_3S$	80
7d	Rust sticky solid	-	$C_{21}H_{27}N_3O_3S$	72
7e	Light brown sticky solid	-	$C_{21}H_{24}ClN_3O_4S$	87
7f	Lemon yellow sticky solid	-	$C_{21}H_{27}N_3O_3S$	89
7g	Dark brown sticky solid	-	$C_{21}H_{27}N_3O_3S$	65
7h	Mustard crystals	139-141	$C_{21}H_{27}N_3O_3S$	89
7i	Buff powder	135-137	$C_{21}H_{27}N_3O_3S$	73
7j	Yellow powder	130-133	$C_{21}H_{27}N_3O_3S$	89
7k	Lemon yellow sticky solid	-	$C_{21}H_{27}N_3O_3S$	67

Table 2: Bioactivity studies of the synthesized compounds

C. No.	BChE		AChE		LOX	
	Inhibition (%) Conc./well (0.5 mM)	**IC_{50}** µM	**Inhibition (%)** Conc./well (0.5 mM)	**IC_{50}** µM	**Inhibition (%)** Conc./well (0.5 mM)	**IC_{50}** µM
6	30.74±0.42	-	39.49±0.21	-	57.96±0.22	329.31±0.08
7a	94.76±0.15	17.11±0.14	94.03±0.71	21.31±0.64	28.33±0.01	-
7b	94.94±0.14	5.31±0.04	62.88±0.54	159.82±0.44	93.75±0.55	183.21±0.06
7c	88.10±0.27	35.41±0.71	51.99±0.31	399.11±0.47	40.94±0.52	-
7d	94.99±0.11	17.62±0.21	76.23±0.19	69.66±0.06	47.61±0.53	-
7e	70.71±0.64	71.21±0.05	65.53±0.01	311.11±0.17	42.09±0.34	-
7f	89.43±0.25	37.31±0.14	49.81±0.37	-	75.77±0.38	225.21±0.31
7g	91.49±0.44	18.31±0.13	79.92±0.38	35.92±0.81	39.46±0.31	-
7h	88.32±0.88	67.91±0.71	51.23±0.54	401.41±0.65	49.94±0.71	-
7i	92.98±0.57	15.91±0.02	88.64±0.92	19.11±0.32	70.28±0.52	236.41±0.95
7j	90.99±0.51	138.61±0.47	75.66±0.64	247.61±0.71	47.22±0.29	-
7k	90.03±0.05	136.91±0.71	90.52±0.85	137.11±0.08	66.84±0.54	188.21±0.15
Control	**Eserine** 82.82±1.09	0.85±0.0001	**Eserine** 91.29±1.17	0.04±0.001	**Baicalein** 93.79±1.27	22.4±1.3

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).
BChE = Butyryl cholinesterase. AChE = Acetyl cholinesterase. LOX = Lipoxygenase.

Enzyme inhibition assays

Cholinesterase assays

The AChE and BChE inhibition assays were done according to the method described by Ellman and coworkers (Ellman *et al.*, 1961) with slight modifications. 100 µL reaction mixture contained 60 µL Na_2HPO_4 buffer having conc. of 50 mM with pH 7.7 was prepared. Test compound of volume ten µL & conc. of 0.5 mM $well^{-1}$ was poured, accompanied by the accession of ten µL enzyme of conc. 0.005 unit $well^{-1}$. All contents were immixed and pre-read at a wavelength of 405 nm. After that pre-incubation of the contents for 10 min at 37ºC was performed and the initiation of the reaction was done through 10 µL of conc. 0.5 mM $well^{-1}$ substrate i.e. acetylthiocholine iodide (for AChE) or butyrylthiocholine chloride (for BChE). Then the ten µL of DTNB with conc. 0.5 mM $well^{-1}$ were added. After incubation of 15 min at 37ºC, absorbance at 405 nm was measured by 96-well plate reader (Synergy HT, Biotek, USA). All the observations were carried out in triplicate with their respective controls. Eserine of conc. 0.5 mM $well^{-1}$ was applied as a positive control. The results were calculated as per formula.

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

IC_{50} values were calculated using EZ–Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA). IC_{50} values were determined by serial dilution of the compounds from 0.5 mM to 0.25, 0.125, 0.0625,

0.03125, 0.015625 mM. IC_{50} value was calculated from the graph, the concentration at which the enzyme inhibition was 50%. Values are mean of 3 independent experiments.

Lipoxygenase assay

Lipoxygenase (LOX) activity was assayed according to the previously described method (Baylac *et al.*, 2003; Bertaccini *et al.*, 1982; Clapp *et al.*, 1985; Kemal *et al.*, 1987) with little variations. 200 µL lipoxygenase assay having 150 µL sodium phosphate buffer of conc. 100 mM with pH 8.0, ten µL compound to be tested and fifteen µL purified lipoxygenase enzyme of conc. 600 units $well^{-1}$ was developed. After mixing, pre-reading at 234 nm and pre-incubation for ten min at 25 °C (room temperature) were processed. Twenty five µL substrate solutions were used for initiation of reaction. The results were based on the change in absorbance, observed after six min at 234 nm using 96-well plate reader (Synergy HT, Biotek, USA). Performance of reaction was done in three-folds. The positive and negative controls were included in the assay. Baicalein (0.5 mM $well^{-1}$) was used as a positive control. The percentage inhibition and IC_{50} values were calculated by the same method as mentioned for cholinesterase assays.

STATISTICAL ANALYSIS

All the measurements were executed in three-folds and statistical analysis was performed by Microsoft Excel (2010). Results are given as mean ± SEM.

RESULTS

The *N*-substituted acetamide derivatives **(7a-k)**, were synthesized according to the protocol sketched in **Scheme-1**. The general reaction conditions and the structure elucidation are described in experimental section.

The parent compound *N*-(Piperidin-1-yl) benzenesulfonamide **(6)** was prepared by the coupling of benzenesulfonyl chloride **(4)** with 1-aminopiperidine **(5)** from the known procedure (Aziz-ur-Rehman *et al.*, 2012 & Aziz-ur-Rehman *et al.*, 2013). Further, the reaction of *N*-(Piperidin-1-yl) benzenesulfonamide **(3)** with different electrophiles **(4a-k)** in the presence of DMF and sodium hydride (NaH) which acts as a base, yielded a series of *N*-aryl/aralkyl substitued-2''-[(phenylsulfonyl) (piperidin-1-yl) amino] acetamides **(7a-k)**. Complete conversion was achieved within 30 to 70 minutes by simple stirring at 50 ^{o}C. The products were isolated by adding cold water in the reaction mixture and filtering off the precipitated solid. In some cases, compound was taken out through solvent extraction method by chloroform/ethyl acetate. All the compounds were spectrally characterized through IR, EI-MS and ^{1}H-NMR analysis.

Enzyme inhibition studies

The findings of *in vitro* enzyme inhibition activity of the synthesized compounds against cholinesterase and lipoxygenase enzymes are presented in **table 1**.

DISCUSSION

Light yellow colored parent compound **(6)** was synthesized in powder form. The molecular formula $C_{18}H_{17}N_3O_3S$ was established *via* EI-MS showing molecular ion peak at *m/z* 240 and counting the number of protons of its ^{1}H-NMRspectrum. The IR spectrum revealed distinguishing peaks at 3430cm^{-1} for SO_2-N-H stretching, 3024 cm^{-1} for C-H str. of aromatic ring, 1546 cm^{-1} for C=C str. of aromatic ring and 1341 cm^{-1} for -SO_2str.of sulfonyl group. The EI-MS gave distinctive peaks at *m/z* 176 and 156 which were ascribed to the loss of SO_2 (sulfonyl) and 1-piperidinyl groups respectively. In the aromatic region of the ^{1}H-NMR spectrum, signals appeared at δ7.90 (dd, *J*=8.7, 1.5 Hz, 2H, H-2' & H-6'), 7.65 (m, 2H, H-3' &H-5') and 7.52 (m, 1H, H-4') which were assigned to the mono substituted benzenesulfonyl ring. In the non-aromatic region of the ^{1}H-NMR spectrum, signals appeared at 2.96 (t, *J*=5.4 Hz, 2H, H_{eq}-2 & H_{eq}-6), 2.47 (t, *J*=5.4 Hz, 2H, H_{ax}-2 & H_{ax}-6), 1.61 (m, 2H, CH_2-4) and1.44 (m, 4H, CH_2-3 & CH_2-5) confirming the presence of piperidine nucleus. Above collective evidences supported the structure of **(6)** which was assigned as *N*-(Piperidin-1-yl) benzenesulfonamide. Similarly, the structure of other compounds was characterized by ^{1}H-NMR, IR and mass spectral data as described in experimental section. The physical data of all the synthesized compounds is shown in **table 1**.

Enzyme inhibition activity

The synthesized compounds exhibited good inhibitory potential against acetylcholinesterase and butyrylcholinesterase as it was evident from their IC_{50} values. It is obvious from **table 2** that compounds *N*-(4-Hydroxyphenyl)-2''-[(phenylsulfonyl)(piperidin-1-yl) amino] acetamide **(7b)** showed good inhibitory potential against butyrylcholinesterase enzyme having IC_{50} value of 5.31±0.04 µmol/L respectively, relative to Eserine, a reference standard with IC_{50} value of 0.85±0.001 µmol/L, probably due to the presence of hydroxy group on the benzene ring in these molecules. The other acetamides which showed good inhibitory potential against butyrylcholinesterase enzyme were **7a**, **7d, 7g** and **7i** having IC_{50} value of 15.91±0.02, 17.11±0.14, 17.62±0.21 and 18.31±0.13 µmol/L respectively. Their good activity may be attributed to the substitution of *N*-(2,6-Dimethylphenyl) acetamide, *N*-(4-Methoxyphenyl) acetamide, *N*-(2-Phenylethyl) acetamide and *N*-(2,4-Dimethylphenyl) acetamide groups, respectively, in these molecules. The enhanced activity might be due to the substitution, which is probably more complimentary for the inhibition of butyrylcholinesterase enzyme. The

screening of acetamides **(7a-k)**, against acetylcholinesterase enzyme depicted that the two compounds, **7a** and **7i**, exhibited good inhibitory potential having IC_{50} 19.11±0.32and 21.31±0.64 µmol/L, relative to Eserine, a reference standard with IC_{50} value of 0.04±0.001 µmol/L. These compounds can further be exploited and their derivatives could be synthesized to acquire closer IC_{50} values of the standard, Eserine. Like this, the compounds could be potential target in the drug discovery and drug development program. However, only few compounds **(Table 1)** found weak activity against LOX enzyme while other remained inactive.

CONCLUSION

The structures of the synthesized bioactive contenders are well supported by spectroscopic data. Most of the synthesized compounds were found to be active against the enzymes as supported by their IC_{50} values. Also our aim of combining amide functionality and piperidine moiety was successful to obtain compounds with effective inhibitory effect. In short, we have inaugurated a new series of compounds with handsome biological activity and the synthesized compounds can be assistive for the pharmaceutical industries in the drug discovery and drug development program.

ACKNOWLEDGMENT

Financial support from Higher Education Commission of Pakistan is highly appreciated.

REFERENCES

Adger B, Dyer U, Hutton G and Woods M (1996). Stereospecific synthesis of the anaesthetic levobupivacaine. *Tetrahedron Lett.*, **37**: 6399-6402.

Aziz-ur-Rehman, Malik A, Riaz N, Nawaz HR, Ahmad H, Nawaz SA and Choudhary MI (2004). Lipoxygenase inhibitory constituents from *Periploca aphylla*. *J. Nat. Prod.*, **67**: 1450-1454.

Aziz-ur-Rehman, Tanveer W, Abbasi MA, Afroz S, Khan KM, Ashraf M and Afzal I (2011). Synthesis, characterization and biological screening of various *N*-substituted derivatives of sulfonamides. *Int. J. Chem. Res.*, **3**(3): 99-104.

Aziz-ur-Rehman, Afroz S, Abbasi MA, Tanveer W, Khan KM, Ashraf M, Ahmad I, Afzal I and Ambreen N (2012). Synthesis, characterization and biological screening of sulfonamides derived from 2-phenylethylamine. *Pak. J. Pharm. Sci.*, **25**: 809-814.

Aziz-ur-Rehman, Awais-ur-Rehman, Abbasi MA, Khalid H, Dar P and Khan KM (2012). Synthesis and biological screening of *N*-substituted derivatives of *N*-benzyl-4-chlorobenzenesulfonamide. *Asian J. Pharm. Hea. Sci.*, **2**: 384-389.

Aziz-ur-Rehman, Rasool S, Abbasi MA, Fatima A, Nafeesa K, Ahmad I and Afzal S (2013). Synthesis, spectral analysis and biological screening of some new *N*-(un) substituted *N*-(5-chloro-2-methoxyphenyl)-aryl sulfonamides. *J. Pharm. Res.,* **6**: 559-564.

Baylac S and Racine P (2003). Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts. *Int. J. of Aromatherap.*, **13**: 138-142.

Bertaccini G and Substance P (1982). Handbook of Experimental Pharmacology, Springer, Berlin, **59**/II, pp. 85-105.

Brau ME, Branitzki P, Olschewski A, Vogeland W and Hempelmann G (2000). Block of neuronal tetrodotoxin-resistant Na^+ currents by stereoisomers of piperidine local anesthetics. *Anesth. Analg.*, **91**: 1499-1505.

Clapp HC, Banerjee A and Rotenberg SA (1985). Inhibition of soybean lipoxygenase by *N*-alkylhydroxylamines. *J. Biochem.,* **24**: 1826-1830.

Cygler M, Schrag JD, Sussman J, Harel LM, Silman I and Gentry MK (1993). Relationship between sequence conservation and three-dimentional structure in a large family of esterases, lipases and related proteins. *Protein Sci.*, **2**: 366-382.

Daly JW and Spande TF (1986). Alkaloids: Chemical and Biological Perspectives. *In*: Pelletier SW editors, Wiley: New York, **4**: pp. 1-254.

Ellman GL, Courtney KD, Andres V and Featherstone RM (1961). A new and rapid calorimetric determination of acetylcholinesterase activity. *Bio. Pharm.*, **7**: 88-95.

Evans AT (1987). Actions of cannabis constituents on enzymes of arachidonate metabolism: Anti-inflammatory potential. *Bio. Pharm.*, **36**: 2035-2037.

Gauthier S (2001). Cholinergic adverse effects of cholinesterase inhibitors in Alzheimer's disease. *Drug Aging*, **18**: 853-862.

Kemal C, Louis-Flemberg P, Krupinski-Olsen R and Shorter AL (1987). Reproductive in activation of soybean lipoxygenase activity. *J. Biochem.,* **26**: 7064-7072.

Kozikowski AP, Araldi GL, Boja J, Meil WM, Johnson KM and Flippen JL (1998). Chemistry and pharmacology of the piperidine-based analogues of cocaine identification of potent DAT inhibitors lacking the tropane skeleton. *J. Med. Chem.*, **41**: 1962-1969.

Nie D and Honn KV (2002). Cyclooxygenase, Lipoxygenase and tumor angiogenesis. *Cell Mol. Life Sci*., **59**: 799-807.

Nithiya S, Karthik N and Jayabharathi J (2011). *In vitro* antioxidant activity of hindered piperidone derivatives. *Int. J. Pharm. Pharm. Sci.*, **3**(3): 254-256.

Roussaki M, Kontogiorgis C A, Litina DH, Hamilakis S and Detsi A (2010). A novel synthesis of 3-aryl coumarins and evaluation of their antioxidant and

lipoxygenase inhibitory activity. *Bioorgan. Med. Chem. Lett.*, **20**: 3889-3892.

Sanchez-Sancho F and Herrandón B (1998). Short syntheses of (*S*)-pipecolic acid, (*R*)-coniine and (*S*)-coniceine using biocatalytically-generated chiral building block. *Tetrahedron Asymmetr.*, **9**: 1951-1965.

Supuran CT, Casini A and Scozzafava A (2003). Protease inhibitors of the sulfonamide type: Anticancer, anti-inflammatory and antiviral agents. *Med. Res. Rev.*, **23**: 535-558.

Tappel AL (1953). The mechanism of the oxidation of unsaturated fatty acid catalyzed by hematin compounds. *Arch. Biochem. Biophys.*, **44**(2): 378-395.

Tougu V (2001). Acetylcholinesterase: Mechanism of catalysis and Inhibition. *Curr. Med. Chem.*, **1**: 155-170.